ANTISENSE OLIGONUCLEOTIDE SEQUENCES DERIVED FROM *groEL* AND *groES* AS INHIBITORS OF MICROORGANISMS

FIELD OF THE INVENTION

The present invention pertains to the field of antimicrobial agents and use thereof in medicine and agriculture.

BACKGROUND

Molecular chaperones are essential to all living organisms (Braig, K., *Curr Opin Struct Biol* (1998) **8**: 159-65). GroEL (HSP60) and GroES (HSP10) are major molecular chaperones and belong to the family of heat shock proteins (HSP). The HSP expression is boosted during cell stress, for example by heat shock, and this helps the cells to survive and to grow at higher temperatures (Buchner, J., *Faseb J* (1996) **10**: 10-9). The proteins are highly conserved in all bacteria (Stamm, et al., *Infect Immun* (1991) **59**: 1572-5; Zeilstra-Ryalls, et al., *Annu Rev Microbiol* (1991) **45**: 301-25).

The precise role of the GroES and GroEL proteins in the bacterial cell is not fully understood, although both proteins are required at all temperatures (Fayet, et al., *J Bacteriol* (1989) **171**: 1379-85; Herendeen, et al., *J. Bacteriology* (1979) **139**: 345-351). GroES and GroEL assist folding of proteins that would otherwise become aggregated or misfolded when refolding from the denatured state (Hartl, F. U. & Martin, J., *Curr Opin Struct Biol* (1995) **5**: 92-102; Hendrick, J. P. & Hartl, F. U., *Annu Rev Biochem* (1993) **62**: 349-84). GroEL and GroES are thought to perform similar protective roles in vivo and thereby increase the half-life of proteins (Hartman, et al., *Proc Natl Acad Sci U S A* (1993) **90**: 2276-80). In *E. coli*, GroEL and GroES are required for the correct assembly of the heads of bacteriophages lambda and T4 (Hendrix, R. W. & Tsui, L., *Proc Natl Acad Sci U S A* (1978) **75**: 136-9). ATP hydrolysis by GroEL results in the release of the bound polypeptides, a process that often requires the action of GroES. (Zeilstra-Ryalls, et al., *J Bacteriol* (1993) **175**: 1134-43). The cooperation of GroEL and GroES is an essential requirement for protein folding (Beissinger, et al., *J Mol Biol* (1999) **289**: 1075-92; Wang, et al., *Proc Natl Acad Sci U S A* (1998) **95**: 12163-8). Mutations in either gene causes similar phenotypes, with respect to lambda phage head morphogenesis and bacterial growth at non-permissive temperatures (Tilly, et al., *Proc Natl Acad Sci U S A* (1981) **78**: 1629-33). Major bacterial HSPs including GroEL have been postulated to possibly contribute to an additional mechanism toward development in bacteria of phenotypic

tolerance to beta-lactam antibiotics (Powell, J. K. & Young, K. D., *J Bacteriol* (1991) **173**: 4021-6).

groEL and *groES* genes are closely linked and are organised in an operon (Hendrick, J. P. & Hartl, F. U., *Annu Rev Biochem* (1993) **62**: 349-84). In prokaryotes, *groEL* codes for the synthesis of approximately 60,000 Mr polypeptide, whereas, *groES*, codes for the synthesis of a small polypeptide of approximately 10,000 Mr (Hendrick, J. P. & Hartl, F. U., *Annu Rev Biochem* (1993) **62**: 349-84; Tilly, et al., *Proc Natl Acad Sci U S A* (1981) **78**: 1629-33).

Escherichia coli has been the most extensively studied model for *groEL* and *groES* transcription. The expression of *groEL* and *groES* mRNA is tightly regulated by a transcriptional activator, the sigma32 subunit of RNA polymerase. Transcription is controlled positively by the level of heat shock-specific sigma32 (Babst, et al., *Mol Microbiol* (1996) **19**: 827-39), and by negative modulators (Tomoyasu, et al., *Mol Microbiol* (1998) **30**: 567-81). At 37 °C the Sigma32 protein is constitutively expressed at a basal level and its level of expression is augmented with temperature (Kanemori, et al., *J Bacteriol* (1994) **176**: 4235-42; Kanemori, M., Mori, H. & Yura, T., *J Bacteriol* (1994) **176**: 5648-53). Sigma32 is itself negatively regulated by the DnaK/DnaJ chaperone system, which inactivates and destabilises sigma32 and FtsH, a membrane-bound metalloprotease that degrades sigma32 (Tatsuta, et al., *Mol Microbiol* (1998) **30**: 583-93; Tomoyasu, et al., *Mol Microbiol* (1998) **30**, 567-81). A conserved regulatory inverted repeat (IR = CIRCE (controlling inverted repeat of chaperone expression)) originally identified in *Bacillus subtilis* functions at the DNA level as a repressor binding site and controls the half life of the transcript (Segal, G. & Ron, E. Z., *J Bacteriol* (1996) **178**: 3634-40; Segal, G. & Ron, E. Z., *Ann N Y Acad Sci* (1998) **851**: 147-51; Segal, R. & Ron, E. Z., *FEMS Microbiol Lett* (1996) **138**: 1-10).

The human Hsp 58 protein represents the equivalent of the bacterial GroEL protein in the mammalian stress protein family (Mizzen, et al., *J Biol Chem* (1989) **264**: 20664-75). In comparison to their human homologues the *groEL* and *groES* genes exhibit sufficient nucleic acid sequence differences.

Depletion of *groEL* appears to be a bacteriocidal rather than a bacteriostatic event, at least at 37 °C (Ivic, et al., *Gene* (1997) **194**: 1-8).

Antibiotics are important pharmaceuticals for the treatment of infectious diseases in a variety of animals including man. The tremendous utility and efficacy of antibiotics results from the interruption of bacterial (prokaryotic) cell growth with minimal damage or

side effects to the eukaryotic host harbouring the pathogenic organisms. In general, antibiotics destroy ba[redacted] by interfering with the DNA replic[redacted], DNA to RNA transcription, translation (that is RNA to protein) or cell wall synthesis.

Although bacterial antibiotic resistance has been recognised since the advent of antimicrobial agents, the consequence of the emergence of resistant microorganisms, such resistance was historically controlled by the continued availability of effective alternative drugs. Now, drug resistance has emerged as a serious medical problem in the community, leading to increasing morbidity and mortality. The problem is worsened by the growing number of pathogens resistant to multiple, structurally unrelated drugs. The situation has become so desperate that antibiotics once removed from use because of toxic effects may be prescribed in an attempt to deal with the otherwise untreatable drug resistant bacteria.

Antisense oligonucleotides have been used to decrease the expression of specific genes by inhibiting transcription or translation of the desired gene and thereby achieving a phenotypic effect based upon the expression of that gene (Wright and Anazodo, *Cancer J.* (1988) 8:185-189). For example, antisense RNA is important in plasmid DNA copy number control, in development of bacteriophage P22. Antisense RNAs have been used experimentally to specifically inhibit *in vitro* translation of mRNA coding specifically from Drosophila hsp23, to inhibit Rous sarcoma virus replication and to inhibit 3T3 cell proliferation when directed toward the oncogene c-fos. Furthermore, it is not necessary to use the entire antisense mRNA since a short antisense oligonucleotide can inhibit gene expression. This is seen in the inhibition of chloramphenicol acetyltransferase gene expression and in the inhibition of specific antiviral activity to vesicular stomatitus virus by inhibiting the N-protein initiation site. Antisense oligonucleotides directed to the macromolecular synthesis operon of bacteria, containing the rpsU gene, the rpoD gene and the dnaG gene have been used for the detection of bacteria. (U.S. Patent No. 5,294,533). Furthermore, photoactivatable antisense DNA complementary to a segment of the β-lactamase gene has been used to suppress ampicillin resistance in normally resistant *E. coli* (Gasparro et al., *Antisense Research and Development* (1991) 1:117-140). Antisense DNA analogues have also been used to inhibit the multiple antibiotic resistance (mar) operon in *E. coli* (White et al., *Antimicrobial Agents and Chemotherapy* (1997) 41:2699-2704).

Accordingly, there remains a need for antisense oligonucleotides which will act to inhibit the growth of microorganisms. Heretofor, no antibiotics or antisense oligonucleotides have been developed to target GroEL or GroES.

This background information is provided for the purpose of making known information believed by the applicant to be of possible relevance to the present invention. No

admission is necessarily intended, nor should be construed, that any of the preceding information constitut[redacted]or art against the present invention. [redacted]lications, patent applications and patents referred to throughout the specification are hereby incorporated by reference in their entireties in this application, as if each individual publication, patent application or patent was specifically and individually indicated to be incorporated by reference in its entirety.

SUMMARY OF THE INVENTION

An object of the present invention is to provide antisense oligonucleotide sequences derived from *groEL* and *groES* as inhibitors of microorganisms. This invention is directed to antisense oligonucleotides which modulate the expression of the groEL and groES molecular chaperones/chaperonin-encoding stress gene in microorganisms and pharmaceutical compositions comprising such antisense oligonucleotides. This invention is also related to methods of using such antisense oligonucleotides for inhibiting the growth of microorganisms.

One aspect of the present invention provides an antisense compound 5 to 50 nucleobases in length targeted to a nucleic acid molecule encoding groEL or groES of a microorganism, wherein said antisense compound specifically hybridises with and inhibits the expression of groES or groES.

One aspect of the present invention provides an antisense compound up to 50 nucleobases in length targeted to a nucleic acid molecule encoding groEL or groES comprising at least a 5 nucleobase portion of SEQ IDNO: 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280,

281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 30[redacted]4, 305, 306, 307, 308, 309, 310, 311[redacted]2, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, or 480 and which inhibits expression of groEL or groES.

Accordingly, in one of its composition aspects, this invention is directed to an antisense oligonucleotide which comprises from about 5 to about 50 nucleotides, which nucleotides are complementary to the *groEL* or *groES* gene of a microorganism. The antisense oligonucleotide may be nuclease resistant. The antisense oligonucleotide may have one or more phosphorothioate internucleotide linkages.

In another of its composition aspects, this invention is directed to an antisense oligonucleotide comprising from about 5 to about 50 nucleotides which is capable of binding to the *groEL* or *groES* gene of a microorganism, wherein the oligonucleotide comprises all or part of a sequence selected from the group consisting of SEQ ID NOs:16-480.

In still another of its composition aspects, this invention is directed to a pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of an antisense oligonucleotide comprising from about 5 to about 50 nucleotides, which nucleotides are complementary to the *groEL* or *groES* gene of a microorganism. The oligonucleotide may be modified, for example, the oligonucleotide may have one or more phosphorothioate internucleotide linkages. Preferably, the antisense oligonucleotide is selected from the group consisting of SEQ ID NOs:16-480.

In one of its method aspects, this invention is directed to a method for inhibiting the expression of the *groEL* gene in a microorganism having a *groEL* gene comprising, administering to said microorganism or to a cell infected with said microorganism an effective amount of an antisense oligonucleotide comprising from at least about 5 nucleotides which are complementary to the *groEL* gene of the microorganism under conditions such that the expression of the *groEL* gene is inhibited.

In another of its method aspects, this invention is directed to a method for inhibiting the expression of the *groES* gene in a microorganism having a *groES* gene, comprising administering to said microorganism an effective amount of an antisense oligonucleotide comprising from at least about 5 nucleotides which are complementary to the *groES* gene of the microorganism under conditions such that expression of the *groES* gene is inhibited.

In one of its method aspects, this invention is directed to a method for inhibiting the growth of a microorganism encoding a *groEL* or *groES* gene, which method comprises administering to said microorganism or a cell infected with said microorganism an effective amount of an antisense oligonucleotide comprising from at least about 5 nucleotides which are complementary to either the *groEL* or *groES* genes of the microorganism under conditions such that the growth of the microorganism is inhibited. Preferably, the antisense oligonucleotide is selected from the group consisting of SEQ ID NOs:16-480.

In another of its method aspects, this invention is directed to a method for treating a pathologic condition in a eukaryotic organism mediated by a microorganism, which method comprises identifying a mammal having a pathologic condition mediated by a microorganism having a *groEL* or *groES* gene and administering to said mammal an effective amount of an antisense oligonucleotide comprising from at least about 5 nucleotides which are complementary to either the *groEL* or *groES* gene of the microorganism under conditions such that the growth of the microorganism is inhibited. Preferably the eukaryotic organism is an animal, more preferably the organism is a mammal.

BRIEF DESCRIPTION OF THE FIGURES AND TABLES

Tables 1, 2, 3 and 4 list exemplary antisense oligonucleotides of the present invention. "Tm" refers to the melting temperature of an oligonucleotide duplex calculated according to the nearest-neighbour thermodynamic values. At this temperature 50% of nucleic acid molecules are in duplex and 50% are denatured. "ΔG" is the free energy of the oligonucleotide, which is a measurement of an oligonucleotide duplex stability. The ΔG for primer stability, hairpin loop and 3' dimer formation has been calculated according to the free energy values (in kcal/mol) of the nearest neighbour nucleotide (Breslauer, et al., *Proc Natl Acad Sci U S A* (1986) **83**, 3746-50). The acceptable ΔG limits for hairpin and 3' dimer formation in Tables 1, 2, 3 and 4 represent a moderate stringency oligonucleotide

selection. Selected conditions: high stability sequences (ΔG>30.0 in absolute number), with minimal internal [redacted] (ΔG>-0.5) and 3' dimer formation ([redacted]>-3.7).

Tables 5 to 8 demonstrate the conservation of some of the oligonucleotide sequences (listed in Tables 1, 2, 3 and 4) among different bacterial organisms. The conserved sequences of oligonucleotides were compared using the BLASTN program (Altschul, et al., *J Mol Biol* (1990) **215**, 403-10) and the National Center for Biotechnology Information (NCBI) databases. "Y" represents 100% of conservation of the oligonucleotide sequence in the corresponding microorganism.

Table 9 lists the different oligonucleotide primers used in the PCR reactions to clone and sequence the *groE* operon from *Escherichia coli*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Figure 1 represents the sequence of the *E. coli groE* operon obtained from GenBank accession number: ECGROESL [SEQ ID NO:1].

Figure 2 represents the sequence of the *E. coli groE* operon of multidrug resistant *Escherichia coli* C175-94 [SEQ ID NO:2].

Figure 3 represents the sequence of the *E. coli groE* operon of the uropathogen ATCC700336 [SEQ ID NO:3].

Figure 4 represents partial sequence of the *E. coli groE* operon from a K12 strain; JM105 [SEQ ID NO:4].

Figure 5 represents the sequence of the *S. pneumoniae groE* operon obtained from GenBank accession number: AF117741 [SEQ ID NO:5].

Figure 6 represents the sequence of the *groE* operon of antibiotic resistant *Streptococcus pneumoniae* isolate ESP174 [SEQ ID NO:6].

Figure 7 represents partial sequence of the *S. pyogenes groEL* gene obtained from GenBank accession number: SPGROELGN [SEQ ID NO:7].

Figure 8 represents partial sequence of the *S. pyogenes groEL* gene of the antibiotic resistant isolate RUH964 [SEQ ID NO:8].

Figure 9 represents partial sequence of *S. pyogenes groEL* gene of the antibiotic resistant isolate RUH969 [SEQ ID NO:9].

Figure 10 represents partial sequence of the *S. pyogenes groEL* gene of the antibiotic resistant isolate RUH983 [SEQ ID NO:10].

Figure 11 represents partial sequence of the *S. pyogenes groEL* gene of the antibiotic resistant isolate RUH1001 [SEQ ID NO:11].

Figure 12 represents the sequence of the *S. aureus groE* operon obtained from GenBank accession number: STAHSP [SEQ ID NO:12].

Figure 13 represents the sequence of the *groE* operon of Methicillin Resistant *Staphylococcus aureus*, isolate B_1318 [SEQ ID NO:13].

Figure 14 represents the sequence of the *groE* operon of *Staphylococcus aureus* RN4220 [SEQ ID NO:14].

Figure 15A and B represent fragments of the sequence of the *groE* operon of *Pseudomonas aeruginosa* ATCC9027 [SEQ ID NO:15].

Figure 16 represents target inhibition using antisense oligonucleotides directed against *groE* operon mRNA.

Figure 17 demonstrates a growth curve of *E. coli* after treatment with antisense oligonucleotide 268a at a concentration of 80 μM.

Figure 18 demonstrates a growth curve of *E. coli* after treatment with antisense oligonucleotide 268b at a concentration of 80 μM.

Figure 19 demonstrates a growth curve of *E. coli* after treatment with antisense oligonucleotide 338 at a concentration of 80 μM.

Figure 20 demonstrates a growth curve of *E. coli* after treatment with antisense oligonucleotide 380 at a concentration of 80 μM.

Figure 21 demonstrates a growth curve of *E. coli* after treatment with antisense oligonucleotide 393 at a concentration of 80 μM.

Figure 22 demonstrates the *in vitro* antimicrobial effect of a methylphosphonate chimeric oligonucleotide (338M) targeting the *groE* operon of *E. coli*.

Figure 23 demonstrates growth curves of *S. aureus* after treatment with antisense oligonucleotide 597 at concentrations of 40 μM (A) and 80 μM (B).

Figure 24 demonstrates growth curves of *S. aureus* after treatment with antisense oligonucleotide 599 at concentrations of 40 μM (A) and 80 μM (B).

Figure 25 demonstrates growth curves of *S. aureus* after treatment with antisense oligonucleotide 600 at concentrations of 40 μM (A) and 80 μM (B).

Figure 26 demonstrates growth curves of *S. aureus* after treatment with antisense oligonucleotide 607 at concentrations of 40 μM (A) and 80 μM (B).

Figure 27 demonstrates a growth curve of *S. aureus* after treatment with antisense oligonucleotide 613 at a concentration of 80 μM.

Figure 28 demonstrates a growth curve of *S. aureus* after treatment with antisense oligonucleotide 622 at a concentration of 80 μM.

Figure 29 demonstrates a growth curve of *S. aureus* after treatment with antisense oligonucleotide 694 at a concentration of 80 μM.

Figure 30 demonstrates a growth curve of *S. aureus* after treatment with antisense oligonucleotide 700 at a concentration of 80 μM.

Figure 31 demonstrates a growth curve of *S. aureus* after treatment with antisense oligonucleotide 708 at a concentration of 80 μM.

Figure 32 demonstrates a growth curve of *S. aureus* after treatment with antisense oligonucleotide 713 at a concentration of 80 μM.

Figure 33 represents t[redacted] *vivo* protection of mice from septic[redacted] using antisense oligonucleotides targeting the mRNA of the *groE* operon. (A) Bacterial counts in the peritoneal cavity of mice treated with phosphorothioate antisense 338 [SEQ ID NO:94]. The peritoneal cavity was flushed with 2 ml of sterile saline solution 2 and 6 hours after infection. Titrated serial dilutions of the lavage fluids were plated on TSA plates (tryptic soy agar, Difco Laboratories), and bacterial counts are given as number of bacteria per millilitre of peritoneal fluid (sample means ± standard deviation, n =3 mice). (B) Bacterial count (CFU/ml) in the peritoneal fluid of mice treated with methylphosphonate chimeric oligonucleotide 338M. Peritoneal fluid was collected under sterile conditions 2 and 7 hours after infection. Titrated serial dilutions of the lavage fluid were plated on TSA plates (tryptic soy agar, Difco Laboratories), and bacterial counts are given as number of bacteria per millilitre of peritoneal fluid (sample means ± standard deviation, n =3 mice). (C) Bacterial count (CFU/ml) in the blood of mice treated with methylphosphonate chimeric oligonucleotide 338M. Blood was collected under sterile conditions. Then 25 µl-aliquots of 10-fold dilutions of blood were plated on TSA plates and incubated under aerobic conditions overnight at 37 ºC. Bacterial counts are given as number of bacteria per millilitre of blood (sample means ± standard deviation, n =3 mice).

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides compounds that inhibit the growth of microbes by inhibiting the expression of a groEL or a groES protein. Without being limited to any theory, the compounds inhibit the expression of the groEL or groES protein by inhibiting the transcription of the *groEL* or *groES* genes or the translation of the mRNA to protein. Such compounds include antisense oligonucleotides.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

The term "antisense oligonucleotide" as used herein means a nucleotide sequence that is complementary to the mRNA for the desired gene. Preferably, the antisense oligonucleotide is complementary to the mRNA for groEL or groES.

The term "*groEL*" refers to any gene which encodes a protein that is also known as HSP60, 60-kDa heat shock protein, cpn60 or Chsp60 in some prokaryotes (Betsou, Sueur & Orfila, 1999; Fink, 1999; LaVerda, Kalayoglu & Byrne, 1999; Richardson, Landry &

Georgopoulos, 1998) and that can also facilitate the actual folding process by providing a secluded environment for individual folding molecules and may also promote the unfolding and re-folding of mis-folded intermediates.

The term "groES" refers to an oligonucleotide sequence which encodes a protein that is also known as HSP10, 10-kDa heat shock protein, cpn10 or Chsp10 in some prokaryotes (Betsou, *et al., 1999; LaVerda* et al., 1999; Richardson *et al.*, 1998) and that facilitate folding protein process in collaboration with the GroEL protein by encapsulating it to form a cis ternary complex, these changes drive the polypeptide into the sequestered cavity and initiate its folding (Sigler *et al.*., 1998).

The term "microorganism" means a bacteria, fungi or virus having either a groEL or groES gene.

The term "bacteria" refers to any bacteria encoding either a groEL or groES gene, including *Escherichi coli*, *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium smegmatis*, *Salmonella typhimurium*, *Thermoplasma acidophilum*, *Pyrococcus furiosus*, *Bacillus subtilis*, *Bacillus firmus*, *Lactococcus lactis, Staphylococcus aureus*, *Staphylococcus carnosus, Listeria monocytogenes*, *Borrelia burgdorferi*, *P. sativum*, *S. griseus*, *Synechoccus sp., Enterococcus faecalis, Streptococcus pneumoniae, Pseudomonas sp., Neisseria meningitidis, Helicobacter pilori and Clostridium difficile.*

The term "virus" refers to any virus having a groEL or groES gene. Preferably the virus will be a DNA virus. Examples of suitable viruses include various herpes viruses (such as herpes simplex types 1 and 2, varicella-herpes zoster, cytomegalovirus and Epstein-Barr virus) and the various hepatitis viruses.

"Sequence homology" refers to the proportion of base matches between two nucleic acid sequences or the proportion amino acid matches between two amino acid sequences. When sequence homology is expressed as a percentage, e.g., 50%, the percentage denotes the proportion of matches over the length from a desired sequence (e.g. *groESL* sequences, such as SEQ ID NOs: 1-15) that is compared to some other sequence. Gaps (in either of the two sequences) are permitted to maximise matching; gap lengths of 15 bases or less are usually used, 6 bases or less are preferred with 2 bases or less more preferred. When using oligonucleotides as probes or treatments the sequence homology between the target nucleic acid and the oligonucleotide sequence is generally not less than 17 target base matches out of 20 possible oligonucleotide base pair matches (85%); preferably not less than 9 matches out of 10 possible base pair matches (90%), and most preferably not less than 19 matches out of 20 possible base pair matches (95%).

"Selectively hybridise[illegible]ers to the ability to detectably and sp[illegible]cally bind. Polynucleotides, oligonucleotides and fragments thereof selectively hybridise to target nucleic acid strands under hybridisation and wash conditions that minimise appreciable amounts of detectable binding to nonspecific nucleic acids. High stringency conditions can be used to achieve selective hybridisation conditions as known in the art and discussed herein. Generally, the nucleic acid sequence homology between the polynucleotides, oligonucleotides, and fragments thereof and a nucleic acid sequence of interest will be at least 30%, and more typically with preferably increasing homologies of at least about 40%, 50%, 60%, 70%, and 90%.

Typically, hybridisation and washing conditions are performed at high stringency according to conventional hybridisation procedures. Positive clones are isolated and sequenced. Typical hybridisation conditions for screening plaque lifts (Benton and Davis (1978) Science 196:180) can be: 50% formamide, 5 x SSC or SSPE, 1-5 x Denhardt's solution, 0.1-1% SDS, 100-200 μg sheared heterologous DNA or tRNA, 0-10% dextran sulfate, I x10^5 to 1 x 10^7 cpm/ml of denatured probe with a specific activity of about I x 10^5 cpm/μg, and incubation at 42°C for about 6-36 hours. Prehybridisation conditions are essentially identical except that probe is not included and incubation time is typically reduced. Washing conditions are typically 1-3 x SSC, 0.1-1% SDS, 50-70°C with change of wash solution at about 5-30 minutes. Cognate sequences, including allelic sequences, can be obtained in this manner.

Two amino acid sequences are homologous if there is a partial or complete identity between their sequences. For example, 85% homology means that 85% of the amino acids are identical when the two sequences are aligned for maximum matching. Gaps (in either of the two sequences being matched) are allowed in maximising matching; gap lengths of 5 or less are preferred with 2 or less being more preferred.

"Corresponds to" refers to a polynucleotide sequence that is homologous (i.e., is identical, not strictly evolutionarily related) to all or a portion of a reference polynucleotide sequence, or to a polypeptide sequence that is identical to all or a portion of a reference polypeptide sequence. In contradistinction, the term "complementary to" is used herein to mean that the polynucleotide sequence is homologous to all or a portion of the complement of a reference polynucleotide sequence. For illustration, the nucleotide sequence "TATAC" corresponds to a reference sequence "TATAC" and is complementary to a reference sequence "GTATA". Preferably the antisense oligonucleotide sequence has at least about 75% identity with the target sequence, preferably at least about 90% identity

and most preferably at least about 95% identity with the target sequence allowing for gaps or mismatches of several bases. Identitiy can be determined, for example, by using the BLASTN program of the University of Wisconsin Computer Group (GCG) software.

The following terms are used to describe the sequence relationships between two or more polynucleotides: "reference sequence," "comparison window," "sequence identity," "percentage of sequence identity," and "substantial identity." A "reference sequence" is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA or gene sequence given in a sequence listing such as SEQ ID NO:1, or may comprise a complete cDNA or gene sequence. Generally, a reference sequence is at least 20 nucleotides in length, frequently at least 25 nucleotides in length, and often at least 50 nucleotides in length. Since two polynucleotides may each (1) comprise a sequence (i.e., a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) may further comprise a sequence that is divergent between the two polynucleotides. sequence, comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity.

A "comparison window", as used herein, refers to a conceptual segment of at least 20 contiguous nucleotide positions wherein a polynucleotide sequence may be compared to a reference sequence of at least 20 contiguous nucleotides and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman (1981) *Adv. Appl. Math.* 2:482, by the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48: 443, by the search for similarity method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci.* (U.S.A.) 85: 2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 573 Science Dr., Madison, WI), or by inspection, and the best alignment (i.e., resulting in the highest percentage of homology over the comparison window) generated by the various methods is selected.

The term "sequence identity" means that two polynucleotide sequences are identical (i.e., on a nucleotide-by-nucleotide basis) over the window of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which

the identical nucleic acid base (e.g., A, T, C, G, U, or I) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 30 percent sequence identity, preferably at least 50 to 60 percent sequence identity, more usually at least 60 percent sequence identity as compared to a reference sequence over a comparison window of at least 20 nucleotide positions, frequently over a window of at least 25-50 nucleotides, wherein the percentage of sequence identity is calculated by comparing the reference sequence to the polynucleotide sequence which may include deletions or additions which total 20 percent or less of the reference sequence over the window of comparison.

The term "inhibiting growth" means a reduction in the growth of the bacteria or viruses of at least 25%, more preferably of at least 50% and most preferably of at least 75%. The reduction in growth can be determined for bacteria by a measuring the optical density of a liquid bacteria culture with a spectrophotometer or by counting the number of colony forming units/ml (CFU/ml) upon plating on culture plates. The reduction in growth can be determined for viruses by measuring the number of plaque forming units/ml upon plating on susceptible cells.

The term "alkyl" refers to monovalent alkyl groups preferably having from 1 to 20 carbon atoms and more preferably 1 to 6 carbon atoms. This term is exemplified by groups such as methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *iso*-butyl, *n*-hexyl, and the like.

The term "aryl" refers to an unsaturated aromatic carbocyclic group of from 6 to 14 carbon atoms having a single ring (e.g., phenyl) or multiple condensed (fused) rings (e.g., naphthyl or anthryl). Preferred aryls include phenyl, naphthyl and the like.

The term "cycloalkyl" refers to cyclic alkyl groups of from 3 to 20 carbon atoms having a single cyclic ring or multiple condensed rings. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like.

The term "halo" or "halogen" refers to fluoro, chloro, bromo and iodo and preferably is either fluoro or chloro.

The term "thiol" refers to the group -SH.

As to any of the above groups which contain one or more substituents, it is understood, of course, that such groups do not contain any substitution or substitution patterns which are sterically impractical and/or synthetically non-feasible. In addition, the compounds of this invention include all stereochemical isomers arising from the substitution of these compounds.

Antisense Oligonucleotides of the Present Invention

It is preferred to target specific nucleic acids for antisense. "Targeting" an antisense compound to a particular nucleic acid, in the context of this invention, is a multistep process. The process usually begins with the identification of a nucleic acid sequence whose function is to be modulated. This may be, for example, a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a nucleic acid molecule from an infectious agent. In the present invention, the target is a nucleic acid molecule encoding bacterial groES or groEL. The targeting process also includes determination of a site or sites within this gene for the antisense interaction to occur such that the desired effect, e.g., detection or modulation of expression of the protein, will result. Within the context of the present invention, a preferred intragenic site is the region encompassing the translation initiation or termination codon of the open reading frame (ORF) of the gene.

The antisense oligonucleotides may be complementary to the complete *groEL* or *groES* genes including the introns. Preferably, the antisense oligonucleotides are complimentary to the mRNA region from the *groEL* or *groES* gene.

In one embodiment of the present invention the antisense oligonucleotides comprise from at least about 5 nucleotides or nucleotide analogues that may be from about 5 to about 50 nucleotides or nucleotide analogues, or from about 7 to about 35 nucleotides or nucleotide analogues or from about 15 to about 25 nucleotide or nucleotide analogues, and further comprise all or part of the sequences set forth in Tables 1 - 4.

The antisense oligonucleotides may be selected from the sequence complementary to the *groEL* or *groES* genes such that the sequence exhibits the least likelihood of showing duplex formation, hair-pin formation, and homooligomer/sequence repeats but has a high to moderate potential to bind to the *groEL* or *groES* gene sequence and contains a GC clamp. These properties may be determined using the computer modelling program OLIGO® Primer Analysis Software, Version 5.0 (distributed by National Biosciences, Inc.,

Plymouth, MN). This computer program allows the determination of a qualitative estimation of these five parameters.

Alternatively, the antisense oligonucleotides may also be selected on the basis that the sequence is highly conserved for either the *groEL* or *groES* genes between two or more microbial species. These properties may be determined using the BLASTN program (Altschul, et al., *J Mol Biol* (1990) **215**, 403-10) of the University of Wisconsin Computer group (GCG) software (Devereux. et al., *Nucleic Acids Res.* (1984) **12**:387-395) with the National Center for Biotechnology Information (NCBI) databases. Preferably the antisense sequence is not conserved in the human groEL or groES genes.

The terms "translation initiation codon" and "start codon" can encompass many codon sequences, even though the initiator amino acid in each instance is typically methionine (in eukaryotes) or formylmethionine (in prokaryotes). It is also known in the art that eukaryotic and prokaryotic genes may have two or more alternative start codons, any one of which may be preferentially utilised for translation initiation in a particular cell type or tissue, or under a particular set of conditions. In the context of the invention, "start codon" and "translation initiation codon" refer to the codon or codons that are used *in vivo* to initiate translation of an mRNA molecule transcribed from a gene encoding groES or groEL, regardless of the sequence(s) of such codons.

The open reading frame (ORF) or "coding region," which is known in the art to refer to the region between the translation initiation codon and the translation termination codon, is also a region which may be targeted effectively. Other target regions include the 5' untranslated region (5'UTR), known in the art to refer to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus including nucleotides between the 5' cap site and the translation initiation codon of an mRNA or corresponding nucleotides on the gene, and the 3' untranslated region (3'UTR), known in the art to refer to the portion of an mRNA in the 3' direction from the translation termination codon, and thus including nucleotides between the translation termination codon and 3' end of an mRNA or corresponding nucleotides on the gene. The 5' cap of an mRNA comprises an N^7-methylated guanosine residue joined to the 5'-most residue of the mRNA via a 5'-5' triphosphate linkage. The 5' cap region of an mRNA is considered to include the 5' cap structure itself as well as the first 50 nucleotides adjacent to the cap. The 5' cap region may also be a preferred target region.

Although some eukaryotic mRNA transcripts are directly translated, many contain one or more regions, known as "introns," which are excised from a transcript before it is translated. The remaining (and therefore translated) regions are known as "exons" and are

spliced together to form a continuous mRNA sequence. mRNA splice sites, i.e., intron-exon junctions, may a[redacted]e preferred target regions, and are p[redacted]ularly useful in situations where aberrant splicing is implicated in disease, or where an overproduction of a particular mRNA splice product is implicated in disease. Aberrant fusion junctions due to rearrangements or deletions are also preferred targets. It has also been found that introns can also be effective, and therefore preferred, target regions for antisense compounds targeted, for example, to DNA or pre-mRNA.

Once one or more target sites have been identified, oligonucleotides are chosen which are sufficiently complementary to the target, i.e., hybridise sufficiently well and with sufficient specificity, to give the desired effect.

In the context of this invention, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics thereof. This term includes oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally-occurring portions which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target and increased stability in the presence of nucleases.

As is known in the art, a nucleoside is a base-sugar combination. The base portion of the nucleoside is normally a heterocyclic base. The two most common classes of such heterocyclic bases are the purines and the pyrimidines. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to either the 2', 3' or 5' hydroxyl moiety of the sugar. In forming oligonucleotides, the phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound. In turn the respective ends of this linear polymeric structure can be further joined to form a circular structure, however, open linear structures are generally preferred. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside backbone of the oligonucleotide. The normal linkage or backbone of RNA and DNA is a 3' to 5' phosphodiester linkage.

Specific examples of antisense compounds useful in this invention include oligonucleotides containing modified backbones or non-natural internucleoside linkages. As defined in this specification, oligonucleotides having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced

in the art, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

Exemplary modified oligonucleotide backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

Representative United States patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Pat. Nos.: 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050.

Exemplary modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

Representative United States patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Pat. Nos.: 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439.

In other oligonucleotide mimetics, both the sugar and the internucleoside linkage, i.e., the backbone, of the nucleotide units are replaced with novel groups. The base units are

maintained for hybridisation with an appropriate nucleic acid target compound. One such oligomeric compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative United States patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat Nos.: 5,539,082; 5,714,331; and 5,719,262. Further teaching of PNA compounds can be found in Nielsen *et al.*, Science, 1991, **254**, 1497-1500.

Exemplary embodiments of the invention are oligonucleotides with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular --CH_2 --NH--O--CH_2 --, --CH_2 --N(CH_3)--O--CH_2 -- [known as a methylene (methylimino) or MMI backbone], --CH_2 --O--N(CH_3)--CH_2 --, --CH_2--N(CH_3)--N(CH_3)--CH_2 -- and --O--N(CH_3)--CH_2 --CH_2 -- [wherein the native phosphodiester backbone is represented as --O--P--O--CH_2 --] of the above referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above referenced U.S. Pat. No. 5,602,240. Also preferred are oligonucleotides having morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

Modified oligonucleotides may also contain one or more substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Particularly preferred are O[$(CH_2)_n$ O]$_m$ CH_3, O$(CH_2)_n$ OCH_3, O$(CH_2)_n$ NH_2, O$(CH_2)_n$ CH_3, O$(CH_2)_n$ ONH_2, and O$(CH_2)_n$ ON[$(CH_2)_n$ CH_3)]$_2$, where n and m are from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2 CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy (2'-O--CH_2 CH_2 OCH_3, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin *et al.*, Helv. Chim. Acta, 1995, **78**, 486-504) i.e., an alkoxyalkoxy group. A further preferred modification includes 2'-dimethylaminooxyethoxy, i.e., a O$(CH_2)_2$ ON$(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples hereinbelow.

Other preferred modifications include 2'-methoxy (2'-O--CH_3), 2'-aminopropoxy (2'-OCH_2 CH_2 CH_2NH_2) and 2'-[redacted]o (2'-F). Similar modifications may [redacted]be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative United States patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos.: 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920.

Oligonucleotides may also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5- hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. I., ed. John Wiley & Sons, 1990, those disclosed by Englisch *et al.*, Angewandte Chemie, International Edition, 1991, **30**, 613, and those disclosed by Sanghvi, Y. S., Chapter 15, Antisense Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B., ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2ºC. (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., eds., Antisense Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Representative United States patents that teach the preparation of certain of the above noted modified nucleosides as well as other modified nucleobases include, but are not limited to, the above noted U.S. Pat. No. 3,687,808, as well as U.S. Pat. Nos.: 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,681,941, and 5,750,692.

Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger *et al.*, Proc. Natl. Acad. Sci. USA, 1989, **86**, 6553-6556), cholic acid (Manoharan *et al.*, Bioorg. Med. Chem. Let., 1994, **4**, 1053-1060), a thioether, e.g., hexyl-S-tritylthiol (Manoharan *et al.*, Ann. N.Y. Acad. Sci., 1992, **660**, 306-309; Manoharan *et al.*, Bioorg. Med. Chem. Lett.,1993, **3**, 2765-2770), a thiocholesterol (Oberhauser *et al.*, Nucl. Acids Res., 1992, **20**, 533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, EMBO J., 1991, **10**, 1111-1118; Kabanov *et al.*, FEBS Lett., 1990, **259**, 327-330; Svinarchuk et al., Biochimie, 1993, 75, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan *et al.*, Tetrahedron Lett., 1995, **36**, 3651-3654; Shea *et al.*, Nucl. Acids Res., 1990, **18**, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, Nucleosides & Nucleotides, 1995, **14**, 969-973), or adamantane acetic acid (Manoharan *et al.*, Tetrahedron Lett., 1995, **36**, 3651-3654), a palmityl moiety (Mishra *et al.*, Biochim. Biophys. Acta, 1995, **1264**, 229-237), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke *et al.*, J. Pharmacol. Exp. Ther., 1996, **277**, 923-937.

Representative United States patents that teach the preparation of such oligonucleotide conjugates include, but are not limited to, U.S. Pat. Nos.: 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single

compound or even at a single nucleoside within an oligonucleotide. The present invention also includes antisense compounds which are chimeric compounds. "Chimeric" antisense compounds or "chimeras," in the context of this invention, are antisense compounds, particularly oligonucleotides, which contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide in the case of an oligonucleotide compound. These oligonucleotides typically contain at least one region wherein the oligonucleotide is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the oligonucleotide may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide inhibition of gene expression. Consequently, comparable results can often be obtained with shorter oligonucleotides when chimeric oligonucleotides are used, compared to phosphorothioate deoxyoligonucleotides hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

Chimeric antisense compounds of the invention may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide mimetics as described above. Such compounds have also been referred to in the art as hybrids or gapmers. Representative United States patents that teach the preparation of such hybrid structures include, but are not limited to, U.S. Pat. Nos.: 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922

The oligonucleotides of the present invention are "nuclease resistant" when they have either been modified such that they are not susceptible to degradation by DNA and RNA nucleases or alternatively they have been placed in a delivery vehicle which in itself protects the oligonucleotide from DNA or RNA nucleases. Nuclease resistant oligonucleotides include, for example, methyl phosphonates, phosphorothioates, phosphorodithioates, phosphotriesters, and morpholino oligomers. Suitable delivery vehicles for conferring nuclease resistance include, for example liposomes.

The oligonucleotides of the present invention may also contain groups, such as groups for improving the pharmacokinetic properties of an oligonucleotide, or groups for improving the pharmacodynamic properties of an oligonucleotide.

In the case of *Mycolplasma smegmatis* (prokaryote), the entry of the antisense oligonucleotide can b[redacted]ilitated by attaching biotin (a labelli[redacted]gent) to the 5' end of the oligonucleotide (Rapaport et al., *Proc Natl Acad Sci USA* (1996) 93:709-713).

The term "pharmaceutically acceptable salt" refers to salts which retain the biological effectiveness and properties of the antisense oligonucleotides of this invention and which are not biologically or otherwise undesirable. In many cases, the antisense oligonucleotides of this invention are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto.

Pharmaceutically acceptable base addition salts can be prepared from inorganic and organic bases. Salts derived from inorganic bases, include by way of example only, sodium, potassium, lithium, ammonium, calcium and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines, such as alkyl amines, dialkyl amines, trialkyl amines, substituted alkyl amines, di(substituted alkyl) amines, tri(substituted alkyl) amines, alkenyl amines, dialkenyl amines, trialkenyl amines, substituted alkenyl amines, di(substituted alkenyl) amines, tri(substituted alkenyl) amines, cycloalkyl amines, di(cycloalkyl) amines, tri(cycloalkyl) amines, substituted cycloalkyl amines, disubstituted cycloalkyl amine, trisubstituted cycloalkyl amines, cycloalkenyl amines, di(cycloalkenyl) amines, tri(cycloalkenyl) amines, substituted cycloalkenyl amines, disubstituted cycloalkenyl amine, trisubstituted cycloalkenyl amines, aryl amines, diaryl amines, triaryl amines, heteroaryl amines, diheteroaryl amines, triheteroaryl amines, heterocyclic amines, diheterocyclic amines, triheterocyclic amines, mixed di- and tri-amines where at least two of the substituents on the amine are different and are selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl, heterocyclic, and the like. Also included are amines where the two or three substituents, together with the amino nitrogen, form a heterocyclic or heteroaryl group.

Examples of suitable amines include, by way of example only, isopropylamine, trimethyl amine, diethyl amine, tri(*iso*-propyl) amine, tri(*n*-propyl) amine, ethanolamine, 2-dimethylaminoethanol, tromethamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, N-alkylglucamines, theobromine, purines, piperazine, piperidine, morpholine, N-ethylpiperidine, and the like. It should also be understood that other carboxylic acid derivatives would be useful in the practice of this invention, for example, carboxylic acid amides, including carboxamides, lower alkyl carboxamides, dialkyl carboxamides, and the like.

Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Salts derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, *p*-toluene-sulfonic acid, salicylic acid, and the like.

Preparation of the Antisense Oligonucleotides of the Present Invention

The antisense oligonucleotides of the present invention may be prepared by conventional and well-known techniques. For example, the oligonucleotides may be prepared using solid-phase synthesis and in particular using commercially available equipment such as the equipment available from Applied Biosystems Canada Inc., Mississauga, Canada. The oligonucleotides may also be prepared by enzymatic digestion of the naturally occurring *groEL* or *groES* genes by methods known in the art.

Isolation and Purification of the Antisense Oligonucleotides

Isolation and purification of the antisense oligonucleotides described herein can be effected, if desired, by any suitable separation or purification such as, for example, filtration, extraction, crystallization, column chromatography, thin-layer chromatography, thick-layer chromatography, preparative low or high-pressure liquid chromatography or a combination of these procedures. However, other equivalent separation or isolation procedures could, of course, also be used.

Although oligonucleotides are taken up by bacterial cells, some modification of the oligonucleotides may help facilitate or regulate said uptake. Thus, a carrier molecule, for example an amino acid, can be linked to the oligonucleotide. For example, bacteria have multiple transport systems for the recognition and uptake of molecules of leucine. The addition of this amino acid to the oligonucleotide may facilitate the uptake of the oligonucleotide in the bacteria and not substantially interfere with the activity of the antisense oligonucleotide in the bacterial cell.

Other methods are contemplated for facilitating the uptake of the antisense oligonucleotide into bacteria. For example, the addition of other amino acids or peptides or primary amines to the 3' or 5' termini of the antisense oligonucleotide may enable utilisation of specific transport systems. Addition of lactose to the oligonucleotide by a covalent linkage may also be used to enable transport of the antisense oligonucleotide by lactose

permease. Other sugar transport systems are also known to be functional in bacteria and can be utilised in this [redacted]ntion.

With regard to inhibiting the expression of groEL or groES in DNA viruses, the antisense oligonucleotide is preferably introduced into the cell infected with the DNA virus. The antisense oligonucleotides may be delivered using vectors or liposomes.

An expression vector comprising the antisense oligonucleotide sequence may be constructed having regard to the sequence of the oligonucleotide and using procedures known in the art. The vectors may be selected from plasmids or benign viral vectors depending on the eukaryotic cell and the DNA virus. Phagemids are a specific example of beneficial vectors because they can be used either as plasmids or a bacteriophage vectors. Examples of other vectors include viruses such as bacteriophages, baculoviruses and retroviruses, DNA viruses, liposomes and other recombination vectors.

Vectors can be constructed by those skilled in the art to contain all the expression elements required to achieve the desired transcription of the antisense oligonucleotide sequences. Therefore, the invention provides vectors comprising a transcription control sequence operatively linked to a sequence which encodes an antisense oligonucleotide. Suitable transcription and translation elements may be derived from a variety of sources, including bacterial, fungal, viral, mammalian or insect genes. Selection of appropriate elements is dependent on the host cell chosen.

Reporter genes may be included in the vector. Suitable reporter genes include β-galactosidase (e.g. lacZ), chloramphenicol, acetyl-transferase, firefly luciferase, or an immunoglobulin or portion thereof. Transcription of the antisense oligonucleotide may be monitored by monitoring for the expression of the reporter gene.

The vectors can be introduced into cells or tissues by any one of a variety of known methods within the art. Such methods can be found generally described in Sambrook et al., 1992; Ausubel et al., 1989; Chang et al., 1995; Vega et al. 1995; and Vectors: A Survey of Molecular Cloning Vectors and Their Uses (1988) and include, for example, stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors.

Introduction of nucleic acids by infection offers several advantages. Higher efficiency and specificity for tissue type can be obtained. Viruses typically infect and propagate in specific cell types. Thus, the virus' specificity may be used to target the vector to specific

cell types *in vivo* or within a tissue or mixed culture of cells. Viral vectors can also be modified with specifi[redacted]eptors or ligands to alter target speci[redacted] through receptor mediated events.

Testing Antisense Oligonucleotides for Anti-groE Effect

The invention contemplates a method of evaluating if an antisense oligonucleotide inhibits the growth of a microbe having a *groEL* or *groES* gene. The method comprises selecting the microbe/microorganism having a *groEL* or *groES* gene present at measurable levels, administering the antisense oligonucleotide; and comparing the growth of the treated microbe with the growth of an untreated microorganism. The effect of the antisense oligonucleotide on expression of the *groES* or *groEL* gene can be readily determined by methods routine in the art, for example Northern blot analysis, ribonuclease protection assays, or RT-PCR.

In order for the antisense oligonucleotide to effectively interrupt the expression of the *groEL* or *groES* gene, the antisense oligonucleotide enters the microorganism's cell, in the case of fungal or bacterial cells or enter the mammalian cell having the virus target.

Pharmaceutical Formulations

When employed as pharmaceuticals, the antisense oligonucleotides are usually administered in the form of pharmaceutical compositions. These compounds can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. These compounds are effective as both injectable and oral compositions. Such compositions are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound.

This invention also includes pharmaceutical compositions which contain, as the active ingredient, one or more of the antisense oligonucleotides associated with pharmaceutically acceptable carriers. In making the compositions of this invention, the active ingredient is usually mixed with an excipient, diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule, sachet, paper or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

In preparing a formulation, it may be necessary to mill the active compound to provide the appropriate particle s[redacted]rior to combining with the other ing[redacted]nts. If the active compound is substantially insoluble, it ordinarily is milled to a particle size of less than 200 mesh. If the active compound is substantially water soluble, the particle size is normally adjusted by milling to provide a substantially uniform distribution in the formulation, e.g. about 40 mesh.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, sterile water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 5 to about 100 mg, more usually about 10 to about 30 mg, of the active ingredient. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient. Preferably, the antisense oligonucleotide is employed at no more than about 20 weight percent of the pharmaceutical composition, more preferably no more than about 15 weight percent, with the balance being pharmaceutically inert carrier(s).

The antisense oligonucleotide is effective over a wide dosage range and is generally administered in a pharmaceutically effective amount. It will be understood, however, that the amount of the antisense oligonucleotide actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

For preparing solid compositions such as tablets, the principal active ingredient/antisense oligonucleotide is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition

may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation is then subdivided into unit dosage forms of the type described above containing from, for example, 0.1 to about 500 mg of the active ingredient of the present invention.

The tablets or pills of the present invention may be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

The liquid forms in which the novel compositions of the present invention may be incorporated for administration orally or by injection include aqueous solutions, suitably flavoured syrups, aqueous or oil suspensions, and flavoured emulsions with edible oils such as corn oil, cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described *supra*. Preferably the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutically acceptable solvents may be nebulized by use of inert gases. Nebulized solutions may be inhaled directly from the nebulizing device or the nebulizing device may be attached to a face mask tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

The following formulation examples illustrate representative pharmaceutical compositions of the present invention.

Formulation Example 1

Hard gelatin capsules containing the following ingredients are prepared:

Ingredient	Quantity (mg/capsule)
Active Ingredient	30.0

Starch	305.0
Magnesium st[redacted]te	[redacted]5.0

The above ingredients are mixed and filled into hard gelatin capsules in 340 mg quantities.

Formulation Example 2

A tablet formula is prepared using the ingredients below:

Ingredient	Quantity (mg/tablet)
Active Ingredient	25.0
Cellulose, microcrystalline	200.0
Colloidal silicon dioxide	10.0
Stearic acid	5.0

The components are blended and compressed to form tablets, each weighing 240 mg.

Formulation Example 3

A dry powder inhaler formulation is prepared containing the following components:

Ingredient	Weight %
Active Ingredient	5
Lactose	95

The active ingredient is mixed with the lactose and the mixture is added to a dry powder inhaling appliance.

Formulation Example 4

Tablets, each containing 30 mg of active ingredient, are prepared as follows:

Ingredient	Quantity (mg/tablet)
Active Ingredient	30.0 mg
Starch	45.0 mg
Microcrystalline cellulose	35.0 mg
Polyvinylpyrrolidone (as 10% solution in sterile water)	4.0 mg
Sodium carboxymethyl starch	4.5 mg
Magnesium stearate	0.5 mg
Talc	1.0 mg
Total	120 mg

The active ingredient, starch and cellulose are passed through a No. 20 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders, which are then passed through a 16 mesh U.S. sieve. The granules so produced

are dried at 50 ºC to 60 ºC and passed through a 16 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 30 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 120 mg.

Formulation Example 5

Capsules, each containing 40 mg of medicament are made as follows:

Ingredient	Quantity (mg/capsule)
Active Ingredient	40.0 mg
Starch	109.0 mg
Magnesium stearate	1.0 mg
Total	150.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 150 mg quantities.

Formulation Example 6

Suppositories, each containing 25 mg of active ingredient are made as follows:

Ingredient	Amount
Active Ingredient	25 mg
Saturated fatty acid glycerides to	2,000 mg

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2.0 g capacity and allowed to cool.

Formulation Example 7

Suspensions, each containing 50 mg of medicament per 5.0 mL dose are made as follows:

Ingredient	Amount
Active Ingredient	50.0 mg
Xanthan gum	4.0 mg
Sodium carboxymethyl cellulose (11%)	
Microcrystalline cellulose (89%)	50.0 mg
Sucrose	1.75 g
Sodium benzoate	10.0 mg
Flavor and Color	q.v.
Purified water to	5.0 mL

The active ingredient, sucrose and xanthan gum are blended, passed through a No. 10 mesh U.S. sieve, and [redacted] mixed with a previously made solut[redacted]f the microcrystalline cellulose and sodium carboxymethyl cellulose in water. The sodium benzoate, flavor, and color are diluted with some of the water and added with stirring. Sufficient water is then added to produce the required volume.

Formulation Example 8

Ingredient	Quantity (mg/capsule)
Active Ingredient	15.0 mg
Starch	407.0 mg
Magnesium stearate	3.0 mg
Total	425.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 425.0 mg quantities.

Formulation Example 9

A formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient	5.0 mg
Corn Oil	1.0 mL

Formulation Example 10

A topical formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient	1-10 g
Emulsifying Wax	30 g
Liquid Paraffin	20 g
White Soft Paraffin	to 100 g

The white soft paraffin is heated until molten. The liquid paraffin and emulsifying wax are incorporated and stirred until dissolved. The active ingredient is added and stirring is continued until dispersed. The mixture is then cooled until solid.

Another preferred formulation employed in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the antisense oligonucleotides of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. See, for example, U.S.

Patent 5,023,252, herein incorporated by reference. Such patches may be constructed for continuous, pulsatile, [redacted] demand delivery of pharmaceutica[redacted]nts.

Another preferred method of delivery involves "shotgun" delivery of the naked antisense oligonucleotides across the dermal layer. The delivery of "naked" antisense oligonucleotides is well known in the art. See, for example, Felgner et al., U.S. Patent No. 5,580,859. It is contemplated that the antisense oligonucleotides may be packaged in a lipid vesicle before "shotgun" delivery of the antisense oligonucleotide.

Frequently, it will be desirable or necessary to introduce the pharmaceutical composition to the brain, either directly or indirectly. Direct techniques usually involve placement of a drug delivery catheter into the host's ventricular system to bypass the blood-brain barrier. One such implantable delivery system used for the transport of biological factors to specific anatomical regions of the body is described in U.S. Patent 5,011,472 which is herein incorporated by reference.

Indirect techniques, which are generally preferred, usually involve formulating the compositions to provide for drug latentiation by the conversion of hydrophilic drugs into lipid-soluble drugs. Latentiation is generally achieved through blocking of the hydroxy, carbonyl, sulfate, and primary amine groups present on the drug to render the drug more lipid soluble and amenable to transportation across the blood-brain barrier. Alternatively, the delivery of hydrophilic drugs may be enhanced by intra-arterial infusion of hypertonic solutions which can transiently open the blood-brain barrier.

Other suitable formulations for use in the present invention can be found in *Remington's Pharmaceutical Sciences* (1985).

The antisense oligonucleotides or the pharmaceutical composition comprising the antisense oligonucleotides may be packaged into convenient kits providing the necessary materials packaged into suitable containers.

Use of the Antisense Oligonucleotides of the Present Invention

The antisense oligonucleotides of the present invention may be used for a variety of purposes. The antisense compounds of the present invention can be utilised for diagnostics, therapeutics, prophylaxis and as research reagents and kits. For therapeutics, they may be used to inhibit the expression of the groEL or groES gene in a microorganism, resulting in the inhibition of growth of that microorganism in an animal,

for example, a human. The animal is treated by administering antisense compounds in accordance with this invention. The compounds of the invention can be utilised in pharmaceutical compositions by adding an effective amount of an antisense compound to a suitable pharmaceutically acceptable diluent or carrier. Use of the antisense compounds and methods of the invention may also be useful prophylactically, e.g., to prevent or delay microbial infection.

The antisense compounds of the invention are useful for research and diagnostics, because these compounds hybridise to nucleic acids encoding groES or groEL, enabling sandwich and other assays to easily be constructed to exploit this fact. Hybridisation of the antisense oligonucleotides of the invention with a nucleic acid encoding groES or groEL can be detected by means known in the art. Such means may include conjugation of an enzyme to the oligonucleotide, radiolabelling of the oligonucleotide or any other suitable detection means. As such, the antisense oligonucleotides of the present invention may also be used to determine the presence of a particular microorganism in a biological sample. Kits using such detection means for detecting the level of groES or groEL in a sample may also be prepared. Finally, the oligonucleotides may be used as molecular weight markers.

In order to further illustrate the present invention and advantages thereof, the following specific examples are given but are not meant to limit the scope of the claims in any way.

EXAMPLES

In the examples below, all temperatures are in degrees Celsius (unless otherwise indicated) and all percentages are weight percentages (also unless otherwise indicated), and the following abbreviations have the following meanings. If an abbreviation is not defined, it has its generally accepted meaning.

µM	=	micromolar
mM	=	millimolar
M	=	molar
ml	=	millilitre
µl	=	microlitre
mg	=	milligram
µg	=	microgram
IPTG	=	isopropyl-β-D-thiogalactoside
PAGE	=	polyacrylamide gel electrophoresis
PVDF	=	polyvinylidene difluoride

rpm	=	revolutions per minute
OD	=	optical density
CFU	=	colony forming units
ΔG	=	free energy, a measurement of oligonucleotide duplex stability
kcal	=	kilocalories

General Methods in Molecular Biology and Microbiology:

Standard microbiological techniques (bacterial growth, titreing of bacterial suspension, etc..) known in the art and not specifically described were generally followed as in Lorian, *Antibiotics in laboratory medicine*. Williams & Wilkins, New York (1996); Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York (1989, 1992); Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore Maryland (1989); and Perbal, *A Practical Guide to Molecular Cloning*, John Wiley & Sons, New York (1988).

The antisense oligonucleotides in Tables 1 - 8 were selected from the sequence complementary to the *groEL* or *groES* genes of various microorganisms such that the sequence exhibited the least likelihood of showing one or more of duplex formation, hair-pin formation, and homooligomer/sequence repeats but had a high to moderate potential to bind to the *groEL* or *groES* gene sequence. These properties were determined using the computer modelling program OLIGO® Primer Analysis Software, Version 5.0 (distributed by National Biosciences, Inc., Plymouth, MN).

A worker skilled in the relevant art would readily appreciate that the antisense oligonucleotides of the present invention may be prepared by various methods known in the art. The phosphorothioate oligonucleotides can be synthesised by companies such as "Sigma Genosys, The Woodlands, Texas". Additionally, a worker skilled in the art would readily appreciate that nucleoside phosphoramidites for oligonucleotide synthesis can be prepared using standard procedures, for example, those outlined in U.S. Patent No. 6,140,126.

Polymerase chain reaction (PCR) was carried out generally as in *PCR Protocols: A Guide To Methods And Applications*, Academic Press, San Diego, CA (1990).

EXAMPLE I

Cloning and sequencing the groE operon from: Escherichia coli, Streptococcus

Streptococcus pneumoniae ESP174 (Simor, A.E., et al., Antimicrob Agents Chemother. (1996) 40: 2190-3) and Streptococcus pyogenes RUH964, RUH969, RUH983 and RUH1001 (Blondeau, J.M., et al., Int J Antimicrob Agents (1999) 12: 67-70) were grown overnight at 37 °C in a 5 % CO_2 atmosphere on columbia agar with 5 % sheep blood (PML Microbiologicals, Mississauga, Ontario, Canada).

Escherichia coli C175-94 (Hvidberg, H., et al., *Antimicrob Agents Chemother.* (2000) **44**: 156-63) and ATCC700336 (Johnson, J.R., et al., *J Infect Dis.* (1997) **175**: 983-8); JM105 (New England Biolabs Inc., Mississauga, Canada); *Staphylococcus aureus* B_1318 (Papakyriacou, H., et al., *J Infect Dis.* (2000) **181**: 990-1000) and RN4220 (Kreiswirth, B.N., et al., *Nature* (1983) **305**: 709-12); and *Pseudomonas aeruginosa* ATCC9027 (Zhang, Y., and R.M. Miller. *Appl Environ Microbiol.* (1992) **58**: 3276-82) were grown overnight at 37 ºC on tryptic soy agar (Difco Laboratories, Detroit, MI, USA) plates.

One or two colonies from each culture were resuspended in 250 µl of water and were heated for 10 min at 100 ºC. Three to five µl from each bacterial suspension was then used to PCR amplify the *groE* operon from *Escherichia coli*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* using primers listed in Table 9.

In order to amplify the fragments containing the *groE* operon, the following conditions were used; after an initial denaturation of target DNA at 95 ºC for 5 min, thermal cycling for each set of primers was 95 ºC for 45 s, 52 ºC for 45 s, and 72 ºC for 45 s for a total of 35 cycles. The final cycle included an extension for 5 min at 72 ºC. Each 100 µl PCR mixture consisted of 30 pmol of each forward and reverse primer, 10 mM Tris-HCl, 1.5 mM MgCl2, 50 mM KCl (pH 8.3; 20 ºC), and 0.75 U of Taq DNA polymerase (Amersham Pharmacia Biotech, Baie d'Urfé, Canada). A deoxyribonucleotide triphosphate mix of dATP, dTTP, dGTP and dCTP (Gibco BRL, Life Technologies, Burlington, Canada) was used at a final concentration of 0.2 mM per reaction mixture. The final PCR products were purified using Quiaquick™ PCR purification kit (Qiagen, Mississauga, Canada) according to the manufacturer instructions. Purified PCR fragments were subjected to double-stranded DNA sequencing with both strands as template by the dideoxyoligonucleotide method of Sanger et al. (Sanger, F., et al., *Proc Natl Acad Sci U S A.* (1997) **74**: 5463-7) at the University of Maine's DNA sequencing facility. DNA sequence analysis was performed using Sequence Navigator software (Applied Biosystems, Inc., Foster City, Calif.), Multalign (Corpet, F., *Nucleic Acids Res.* (1988) **16**:

10881-90) and the basic local alignment search tool (BLAST) (Altschul, S.F., et al., *J Mol Biol.* (1990) 215: 403[redacted]at the National Library of Medicine,[redacted]onal Institutes of Health.

Results indicated that the genomic sequence of the *groE* operon is highly conserved (≥90%) in each bacterial species; in ATCC strains, field isolates and antibiotic resistant strains. Furthermore, stretches of DNA sequences are conserved between the *groE* operon sequences of pathogens of different species, genus and families.

EXAMPLE II

Messenger RNA target inhibition using antisense oligonucleotides directed against *groE* operon mRNA

RT-PCR is a semi-quantitative gene expression analysis technique that was used to detect *in vitro* mRNA target inhibition. Bacteria treated with anti-groE antisense oligonucleotides demonstrated a reduction of groE mRNA in comparison to untreated bacteria.

Electrocompetent *E. coli* K12 were transformed with 200 μM of each phosphorothioate antisense oligonucleotide. Approximately 10^{10} CFU/ml were electroporated using a Cell Porator (Gibco BRL, Burlington, Canada) in micro electro-chambers (0.4 cm between the electrodes) at a pulse of 2.4 kV, 4 kΩ. Control populations of bacteria were subjected to electroporation but without antisense oligonucleotides. Messenger RNA was immediately isolated using RNeasy MiniKit™ (Qiagen, Mississauga, Ontario) according to the manufacturer's instructions.

One hundred and fifty ng of each of the purified total RNA were reverse transcribed into cDNA using the Ready-To-Go™ You-Prime First-Strand Beads (Amersham Pharmacia Biotech, Baie d'Urfé, Canada) according to the manufacturer's instructions. Thirty pmol of the primer secaRe (5'-CACCTTCTTTCGCTTCCAC-3' [SEQ ID NO:503]) for the mRNA of house keeping negative control and 30 pmol of the primer groeRe (5'-AGATTTTCTTGTCAGCCAGCA-3' [SEQ ID NO:504]) for the targeted *groE* operon mRNA were used for the reverse transcription. The following conditions were used to amplify the cDNA of the *groE* operon, or the *secA* house keeping gene; after an initial denaturation of target cDNA at 95 ºC for 5 min, thermal cycling for each set of primers was 95 ºC for 45 s, 51 ºC for 45 s, and 72 ºC for 45 s for a total of 35 cycles. The final cycle included an extension for 5 min at 72 ºC. The 100 μl PCR mixtures consisted of 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl (pH 8.3; 20 ºC), and 0.75 U of Taq DNA

polymerase (Amersham Pharmacia Biotech, Baie d'Urfé, Canada). Primers to amplify the *secA* cDNA (forward: [redacted]GTTACCGTCAACGACTACC-3' [SEQ ID NO:505]and reverse: 5'-CACCTTCTTTCGCTTCCAC-3' [SEQ ID NO:506]) and the *groE* cDNA (forward: 5'-GAGTTATCAATGAATATTCGTCC-3' [SEQ ID NO:507] and reverse: 5'-AGATTTTCTTGTCAGCCAGCA-3' [SEQ ID NO:508]) were each used at a final concentration of 30 pmol per reaction mixture.

Final analysis was done by applying PCR products, 15 µl/well on agarose gels containing 1.5% (wt/vol) agarose (Gibco BRL) prepared and run in 1× TAE buffer [1× TAE is 40 mM Tris-acetate [pH 8.0], 1 mM EDTA] and separated by electrophoresis. DNA in the gel was stained with ethidium bromide (5 µg/ml) and visualised and photographed under UV light.

Figure 16 represents the electrophoretogram of mRNA obtained from *E. coli* bacteria treated with antisense oligonucleotides 357 and 338. In each case the antisense oligonucleotide treatment resulted in a decrease in mRNA.

Results indicated that antisense oligonucleotides are specifically inhibiting the mRNA of the *groE* operon of *E. coli*. There is no reduction in expression of the mRNA copy number of the selected house keeping gene (*secA*) by antisense oligonucleotides targeting the mRNA of the *groE* operon of *E. coli*.

EXAMPLE III

In vitro antimicrobial effect on a Gram negative *E. coli* and a Gram positive, *S. aureus* of anti-groE antisense oligonucleotides

E. coli (K12) or *S. aureus* (RN4220) were electroporated with either 80 µM or 40 µM of phosphorothioate or methylphosphonate antisense oligonucleotides. Approximately 10^{10} CFU/ml were electroporated using a Cell Porator (Gibco BRL, Burlington, Canada) in micro electro-chambers (0.4 cm between the electrodes) at a pulse of 2.4 kV, 4 kΩ. Control populations were subjected to electroporation in the absence of antisense oligonucleotides. Equal numbers of the treated or untreated *E. coli* or *S. aureus* cells were then transferred to flasks containing fresh Luria-Bertani broth (Miller J. H. Experiments in Molecular Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1972)) and grown at 30 ºC on a shaker at 250 rpm. The number of bacteria per flask was determined by the turbidity of the cultures at OD_{620} measured each hour (Carpentier P.L., Microbiology 4th ed. W.B. Saunders Company (1977)).

Figures 17 to 21 show the rate growth of the *E. coli* following treatment with various phosphorothioate antisense oligonucleotides. Figure 22 shows the rate of growth of *E. coli* following treatment with a methylphosphonate chimeric antisense oligonucleotide. Figures 23 to 32 show the growth rate of *S. aureus* following treatment with the various antisense oligonucleotides.

The results indicated that antisense oligonucleotides targeting the mRNA of the *groE* operon of *E. coli* or *S. aureus*, significantly inhibit bacterial growth in a dose response effect and that this antimicrobial activity is not dependent on the structure of the backbone of the antisense oligonucleotide.

EXAMPLE IV

Protection of mice from septicemia using antisense oligonucleotides targeting the mRNA of the *Escherichia coli groE* operon

Chimeric oligonucleotide 338M and phosphorothioate oligonucleotide 338 efficacy compared to a negative control were evaluated *in vivo* in an animal model of septicemia (Barekzi, N.A., et al., *Antimicrob Agents Chemother.* (1999) **43**: 1609-15; Fridmodt-Møller, N., et al., *In* Handbook of animal models of infection. O. Zak and S.M. A., editors. Academic Press, London. (1999) 127-136; Zantl, N., et al., *Infect Immun.* (1998) **66**: 2300-9) in two independent experiments (see Figure 33A and B)

338M: 2'*OMe* (CCG) *p*(AATTTTACGTCTTT) 2' *OMe* (AGC), where *OMe* represents methylphosphonates nucleotides and p represents a phosphodiester linkage oligonucleotide backbone. 338: all phosphorothioate linkage oligonucleotide backbone (5'-CCGAATTTTACGTCTTTAGC-3' [SEQ ID NO:94])

Challenge

Prior to bacterial challenge, mice were kept for at least 1 week in the animal facility to recover after shipment. All experimental procedures were performed according to the Animal Care Committee of the Sunnybrook & Women's College Health Science Centre. Female CD1 mice, 7 weeks old (Charles River, Wilmington, Mass.) were infected intraperitonealy with 500 µl of a bacterial suspension of 10^7-10^8 CFU/ml of *Escherichia coli* (ATCC25922) resuspended in phosphate buffered saline pH 7.4.

Treatment:

Phosphorothioate and chimeric antisense molecules were resuspended in PBS (phosphate buffered saline) pH 7.4 at a concentration of 2.5 mg/ml. Two hundred µl of the test drug or

saline (control group) were injected intraperitoneally at 1 and 3 hours post infection with antisense 338. In another independent experiment, 200 µl of the drug or saline (control group) were injected intraperitoneally at 1 and 4 hours post infection with antisense 338M.

Monitoring of the infection level:

At various times postinfection (2 and 6 hours with phosphorothioate antisense 338 and 2 and 7 hours with chimeric phosphorothioate antisense 338M), 10 µl of mice blood was withdrawn from a cut in the lateral tail vein from three mice by group by time point. After the mice were euthanatized (via cervical dislocation), a saline lavage of the peritoneal cavity was performed by using 2 ml of sterile saline, and lavage fluid (~0.5 ml) was collected. Blood and peritoneal lavage fluid were serially diluted and plated on tryptic soy agar plates (Difco Laboratories), and bacterial colonies were enumerated after 18 h of incubation at 37°C.

Results indicate that antisense oligonucleotides with 2 different backbones targeting the mRNA of the *groE* operon of *E. coli* can significantly reduce the infectious load in vivo in the antisense treated group compared to the saline control group in a mice model of septicemia. A significant $\log_{10}$ reduction in CFU/ml of ≥ 1.5 was achieved 6 hours after bacterial challenge in peritoneal fluid of mice treated with antisense phosphorothioate 338 targeting the mRNA of the *groE* operon of *E. coli*. In mice treated with methylphosphonate chimeric antisense oligonucleotide (338M) a greater log10 reduction in CFU/ml of ≥ 3 was achieved 7 hours after bacterial challenge in peritoneal fluid. Furthermore, in mice treated with methylphosphonate chimeric antisense oligonucleotide (338M) a significant log10 reduction in CFU/ml of ≥ 2.5 was achieved 7 hours after bacterial challenge in peripheral blood. In the experiment where mice were treated with antisense phosphorothioate 338, the level of septicemia in the peripheral blood of the control and treated mice groups was too low to be included in the results.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the appended claims.

Table 1: Antisense Oligonucleotides that target *Escherichia coli groE* operon

SEQ ID No:	Name	Sequence 5' → 3'	Tm (°C)	ΔG (kcal/mol)
16	266	GGGGGAAAAAGAAAAAAAAT	48.9	39.6
17	267	TCAAGGGGGAAAAAGAAAAA	50.8	39.4
18	268	CAGAGAAATGGGGATGGCTT	62.7	45.5
19	268a	AATGGACGAATATTCATTGA TAACTCTCCTTTGAGAAAGT CCGTATCTGT	84.6	87.2
20	268b	TATTCATTGATAACTCTCCT	47.0	31.6
21	268c	TGCAATGGACGAATATTCAT	57.1	36.2
22	269	ACTTCTTTACGCTTGACGAT	46.0	35.7
23	270	AACTTCTTTACGCTTGACGA	47.2	36.1
24	271	CCGCCAGCAGATTTAGTTTC	51.8	39.1
25	272	GACAGCCAGCACTTCGCCGC	62.6	44.0
26	273	CGCCATTTTCAAGGATACGG	54.6	41.0
27	274	GCTTCACTTCGCCATTTTCA	52.9	38.9
28	275	GCCAACTTTCACATCCAGCG	55.5	39.8
29	276	GTAGCCATCGTTGAAAATAA	44.9	35.3
30	277	CTCAGATTTCACACCGTAGC	47.2	35.2
31	278	CGTATGTTCAGTGTCGTGCG	52.4	37.1
32	279	TGCCATTATCTTTATTCCTT	43.8	35.3
33	279a	CTGCCATTATCTTTATTCCT	52.8	35.2
34	279b	TTTACGTCTTTAGCTGCCAT	56.7	36.8
35	280	CGCAGCATTTTCACACGAGC	56.7	40.3
36	281	CCGAGGGTAACTTTCACTGC	51.4	38.3
37	282	TCTTTGGTGATGGTCGGTGC	55.6	39.2
38	283	ACGAGCAACGGAAACACCAT	54.3	39.4
39	284	GCAACTTCTTTCACCATCTG	47.1	35.1
40	285	GCTTTAGAGGCAACTTCTTT	45.3	36.0
41	286	GCGTCGTTTGCTTTAGAGGC	54.3	40.6
42	287	AGTGATGATAGCCTGAGCCA	49.0	36.4
43	288	TTCAGGTCCATCGGGTTCAT	54.0	39.1
44	289	GCAATCGCTTTAGAGTCAGA	47.3	35.8
45	290	CGTCGGAGTTAGCGGAGATG	54.8	40.1
46	291	TTCGTCGGAGTTAGCGGAGA	54.9	40.2
47	292	CGGTTTCGTCGGAGTTAGCG	57.1	42.2

SEQ ID No:	Name	Sequence 5 → 3	Tm (ºC)	ΔG (kcal/mol)
48	293	ACGCCTTCTTTACCGACTTT	50.1	38.8
49	294	ACGCCTTCTTTACCGACTT	48.3	36.9
50	295	TAACGCCTTCTTTACCGACT	48.7	37.8
51	296	GATAACGCCTTCTTTACCGA	49.0	38.0
52	297	GTGATAACGCCTTCTTTACC	46.0	36.0
53	298	AAGTAAGGAGACAGGTAGCC	44.5	35.0
54	299	TTGATGAAGTAAGGAGACAG	41.3	32.3
55	299a	AGGAG	-	-
56	300	CAGCCAGCAGGATGAACGGG	60.0	42.5
57	301	GATTTTCTTGTCAGCCAGCA	50.3	36.9
58	302	CCTTCTACATCTTCAGCGAT	46.1	35.4
59	303	CGCTTCGCCTTCTACATCTT	51.5	39.0
60	304	CAGCGACTTTCACGATGCCA	57.0	40.0
61	305	TCCAGGGTTGCTTTTTCCAG	54.2	40.0
62	306	CCAGGTCTTCCAGGGTTGCT	55.4	40.3
63	307	GCTTCTTCACCCACGCCATC	56.8	40.7
64	308	CGGTCGTAGTCAGAAGTTGC	49.9	36.7
65	309	AGCACCCACTTTGATAACTG	46.7	35.3
66	310	TTCAACTTCGGTAGCAGCAC	50.5	37.1
67	311	TTCTCTTTCATTTCAACTTC	41.0	32.7
68	312	TTTCTCTTTCATTTCAACTT	40.9	33.0
69	313	TTTTCTCTTTCATTTCAACT	40.9	33.0
70	314	TTTTTCTCTTTCATTTCAAC	41.4	33.3
71	315	TTTTTTCTCTTTCATTTCAA	42.2	33.9
72	316	CTTTTTTCTCTTTCATTTCA	41.7	33.6
73	317	GCTTTTTTCTCTTTCATTTC	43.2	34.8
74	318	CTTTTTTCTCTTTCATTTC	38.3	31.7
75	319	TGCTTTTTTCTCTTTCATTT	44.0	35.1
76	320	CCTTCTTCTACCGCAGCACG	50.0	40.3
77	321	CCACGCCTTCTTCTACCGCA	57.5	41.8
78	322	GCAACACCACCACCAGCAAC	56.0	38.8
79	323	CTTCGCCGCAGTTCAATACG	55.8	41.0
80	324	AGTTGCCGTCGCCGCCTTTA	45.3	61.3
81	325	ACCGTAGTTGCCGTCGCCGC	63.8	46.1
82	326	TTCTTCGGTTGCTGCGTTGT	55.7	40.1

SEQ ID No:	Na[redacted]	Sequence 5 → 3'	T[redacted] (ºC)	ΔG (kcal/mol)
83	327	ACGAGTTACTTTGGTTGG	41.8	32.1
84	328	TTTTTCGGCAGGTCGGTAAC	54.2	40.6
85	329	ATGCCGCCCATACCGCCAGC	65.5	47.5
86	330	CGGGGGTTTGTTTATTTCTG	51.4	39.4
87	331	CGATCATGCAATGGACGAAT	53.2	38.5
88	332	TCTTTACGCTTGACGATCAC	47.8	35.7
89	333	TTTCAACTTCTTTACGCTTG	45.3	35.3
90	334	AGAACGATGCCGCCAGCAGA	60.3	42.7
91	335	CCGGTCAGAACGATGCCGCC	64.2	45.6
92	336	AATTGCCAGAATGTCGCTTT	51.6	38.8
93	337	TTTACGTCTTTAGCTGCCAT	47.2	36.6
94	338	CCGAATTTTACGTCTTTAGC	46.7	37.1
95	339	ACCGAATTTTACGTCTTTAG	43.6	35.3
96	340	TTACCGAATTTTACGTCTTT	44.1	35.6
97	341	TCGTTACCGAATTTTACGTC	47.3	36.7
98	342	ACGTTACGGCCTTTTGGACC	55.4	41.5
99	343	GAAACACCATCTTTGGTGAT	46.1	34.7
100	344	GTCTTCCAGTTCGATTTC	40.7	31.5
101	345	TTGTCTTCCAGTTCGATTTC	46.7	35.3
102	346	ACTTGTCTTCCAGTTCGATT	45.2	34.7
103	347	AACTTGTCTTCCAGTTCGA	44.1	33.2
104	348	CGAACTTGTCTTCCAGTTCG	49.8	36.8
105	349	TCGAACTTGTCTTCCAGTTC	46.5	34.8
106	350	TTTTCGAACTTGTCTTCCAG	47.1	35.7
107	351	CATATTTTCGAACTTGTCTT	41.3	33.3
108	352	CCATATTTTCGAACTTGTCT	43.7	34.5
109	353	GCACCCATATTTTCGAACTT	48.6	37.5
110	354	TTCATGCCCGCAGCAACAGC	61.6	43.1
111	355	TCCATCGGGTTCATGCCCGC	64.4	45.6
112	356	GTCCATCGGGTTCATGCCCG	61.7	43.8
113	357	AGGTCCATCGGGTTCATGCC	58.2	41.8
114	358	CAGGTCCATCGGGTTCATGC	57.3	40.6
115	359	TCAGGTCCATCGGGTTCATG	55.3	39.1
116	360	TAACCGCTTTGTCGATACC	47.8	36.1
117	361	GCGGTAACCGCTTTGTCGAT	56.5	41.9

SEQ ID No:	[redacted]	Sequence 5 → 3'	[redacted] (ºC)	ΔG (kcal/mol)
118	362	AACTGCATACCTTCAACCAC	46.5	35.0
119	363	TGCATACCTTCAACCAC	40.4	30.2
120	364	GAACTGCATACCTTCAACCA	47.4	35.3
121	365	CGAACTGCATACCTTCAACC	49.7	37.0
122	366	AACTGCATACCTTCAAC	35.9	28.7
123	367	CGGTCGAACTGCATACCTTC	52.2	38.4
124	368	TCAGCGATGATCAGCAGCGG	59.1	41.4
125	369	GCGATGATCAGCAGCGG	53.1	36.3
126	370	TTCAGCGATGATCAGCAGCG	57.2	40.2
127	371	CTTCAGCGATGATCAGCAGC	53.4	38.2
128	372	TCTTCAGCGATGATCAGCAG	51.2	36.7
129	373	TTACGACGATCGCCGAAGCC	59.6	43.6
130	374	TTTACGACGATCGCCGAAGC	57.6	42.4
131	375	CTTTACGACGATCGCCGAAG	54.9	40.9
132	376	GCTTTACGACGATCGCCGAA	57.6	42.4
133	377	AGCATAGCTTTACGACGATC	46.6	36.1
134	378	CAGCATAGCTTTACGACGAT	47.5	36.4
135	379	ACGCGTTCCTGCAGTTTTTC	54.4	40.1
136	380	TTTGATAACTGCAACGCC	46.7	34.4
137	381	CGCAGCGGAGCTTCCATTGC	62.5	44.8
138	382	ACGATCTGACGCAGCGGAGC	59.9	42.3
139	383	ATCATGTTGCCGTATTCTTC	46.7	35.6
140	384	GTCGATCATGTTGCCGTATT	49.9	37.0
141	385	TACTGCAGAGCAGAACGAGT	47.4	35.0
142	386	GCGTACTGCAGAGCAGAACG	53.6	38.5
143	387	CATGCATTCGGTGGTGATCA	54.8	38.1
144	388	TGCCGCCCATACCGCCAGCA	67.9	48.0
145	389	CCATGCCGCCCATACCGCCA	67.1	47.9
146	390	CCACCCATGCCGCCCATACC	63.6	45.6
147	391	ATGCCACCCATGCCGCCCAT	65.8	46.8
148	392	CATGCCACCCATGCCGCCCA	68.3	47.2
149	393	CCATGCCACCCATGCCGCCC	68.7	48.4
150	394	CCCATGCCACCCATGCCGCC	68.7	48.4
151	395	CCGCCCATGCCACCCATGCC	68.7	48.4
152	396	GCCGCCCATGCCACCCATGC	68.9	48.4

SEQ ID No:	N[redacted]	Sequence 5 → 3'	[redacted] (°C)	ΔG (kcal/mol)
153	397	ATGCCGCCCATGCCACCCAT	65.8	46.8
154	398	CATGCCGCCCATGCCACCCA	68.3	47.2
155	399	TCATGCCGCCCATGCCACCC	67.4	46.9
156	400	CATCATGCCGCCCATGCCAC	63.7	44.1
157	401	TACATCATGCCGCCCATGCC	61.0	43.1
158	402	TTACATCATGCCGCCCATGC	58.8	41.9
159	403	TATTTCTGCGAGGTGCAG	46.7	34.1

Table 2: Antisen[redacted]ligonucleotides that target *Strepto[redacted]us pneumoniae groE* operon

SEQ ID NO:	Name	Sequence 5 → 3'	Tm (ºC)	ΔG (kcal/mol)
160	404	CGCATAGCACGTTGGGCACG	61.8	43.7
161	405	GACCAAATCTGCCAAATCTT	48.4	36.8
162	406	GGCAATTCTTCCTCTTCCAA	51.0	38.4
163	407	AAGATAAAAAGCCTGAGACC	44.8	35.6
164	408	GGTCAGAAATAGTCAAGAAA	40.8	32.5
165	409	ATTCTATTGTATCACTTGGT	37.5	31.1
166	410	TGGAGTGCTAATTCATAATT	41.7	33.5
167	411	TTAGCACTCTTTGAACTGGA	44.9	34.2
168	411a	TCAACATCTGATTTCCTCCA	56.4	35.0
169	411b	TCCCCTAATGGTTTCAACAT	57.9	37.1
170	412	CGGTCCCCTAATGGTTTCAA	53.8	40.3
171	413	GCACACGGTCCCCTAATGGT	55.9	40.6
172	414	TAAGAGCACACGGTCCCCTA	52.2	38.5
173	415	AACGGTTTGTTCTTTTTCTT	45.4	36.0
174	416	TGGTTTTTTCTTGGGCTGAG	52.0	38.8
175	417	GCTGTTTTGGTTTTTTCTTG	47.1	36.4
176	418	CCTGCGTGGGCTTCAACTAA	55.2	40.4
177	419	ACATCAAGACCTGCGTGGGC	57.1	40.4
178	420	TACTTTTCATCGCCATCTTT	46.8	36.3
179	421	CTTTCTCCTTCTATTCCTCA	42.8	34.1
180	421a	TGACATACTTACTTTCTCCT	46.8	31.2
181	422	ATTTCTTTTGACATACTTAC	35.3	30.3
182	422a	AATTTAATTTCTTTTGACAT	47.7	32.5
183	423	ATATCATTGGTTTTAGAAGC	40.8	33.5
184	424	CGTAGTCCCGTCACCTGCGA	58.2	41.1
185	425	AATCCCACGACGAATACCGA	54.2	40.0
186	426	ACTGCTGTTTCAATCCCACG	52.4	38.0
187	427	CAGGGATGGCGTTGTTTTTC	54.4	40.1
188	428	TCAGAACGAGAAGATACGGC	49.5	36.9
189	429	TCCATTGCTTCAGAGATGTA	45.3	34.0
190	430	ACTTTTTCCATTGCTTCAGA	46.7	35.7
191	431	AACTTTTTCCATTGCTTCAG	46.5	36.0
192	432	CCAACTTTTTCCATTGCTTC	49.2	37.5

SEQ ID NO:	N[redacted]	Sequence 5 → 3' [redacted]	Tm (°C)	ΔG (kcal/mol)
193	433	TTGCCAACTTTTTCCATTGC	52.8	39.3
194	434	GCATTCCTTCTACGACTTCA	46.9	35.5
195	435	TAACCACGGTCAAACTGCAT	50.4	37.0
196	436	CCATTTTTTCGCTATCAG	43.1	33.4
197	437	GTCAGCCACCATTTTTTCGC	54.6	40.1
198	438	CGGATTTTCAAGGTCAGCCA	55.0	40.1
199	439	TGGACGATTGCTTTGGAGAA	53.1	38.6
200	440	CATCCGCAATAATCAAGAGT	46.9	35.9
201	441	CCATCCACATCATCCGCAAT	55.3	39.5
202	442	CCTCGCCATCCACATCATCC	57.3	40.5
203	443	GGTGCCTTGACTGCTACTAC	46.4	35.0
204	444	CAAGCATGGCTTTGCGACGG	61.0	43.6
205	445	TGTCGCATCTTTCAACTCAA	49.0	35.6
206	446	TCCACGGTCACTCTCGCTGC	58.4	40.4
207	447	AACCGTGCTATCTTTGTCCA	49.7	37.0
208	448	GCACCTTCTACAATAACCGT	46.2	35.7
209	449	TTTGAGACTTGATAACCGCA	48.3	36.1
210	450	CCGCTACACCACCTGACAAT	52.3	37.9
211	451	TAATAACCGCTACACCACCT	46.7	36.2
212	452	CAGTTTCAGTTGCGGCTCCA	56.1	39.9
213	453	CGGAGTTTCATTTCTTTCAA	47.2	36.2
214	454	ATCTTCAATGCGGAGTTTCA	49.7	37.0
215	455	GTAGCGTTGAGGGCATCTTC	51.4	38.2
216	456	ACAATACCTTCTTCAACAGC	43.4	33.8
217	457	AATACCTTCTTCAACAGC	38.2	30.6
218	458	AACAATACCTTCTTCAACAG	40.9	32.6
219	459	GCAACAATACCTTCTTCAAC	43.9	34.1
220	460	CGTCCTGTTGCTTCATCTCC	52.0	37.7
221	461	CCAAAGCACGGAGAACAATA	50.4	37.6
222	462	ATTTTGTAGGGCTGAACGAC	48.6	37.0
223	463	GCTGGGGCTACTGGTTCTGG	56.0	40.8
224	464	TCCCGCCCATCATGCTTGGA	62.8	44.2
225	465	TTACATCATCCCGCCCATCA	55.9	40.1
226	466	CCTCCTTTTGTGTTTTTT	41.5	32.6
227	467	TCTTTTTCTTCTATTTT	29.8	27.2
228	468	ATCGCCATCTTTGACATCAA	50.3	36.9

SEQ ID NO:	N[redacted]	Sequence 5 → 3' [redacted]	Tm (ºC)	ΔG (kcal/mol)
229	469	TCTTCCAATTCGATTTCTTT	45.9	36.1
230	470	GGTCTTCCAATTCGATTTCT	47.5	36.7
231	471	CCGTCACCTGCGATATCATT	52.4	38.5
232	472	GGATTTGCACCTGCTGTGA	51.6	36.2
233	473	TCTGTTTCCATACCACGTGA	48.4	35.2
234	474	TCAAACTGCATTCCTTCTAC	44.3	34.1
235	475	TCAAGGTCAGCCACCATTTT	52.0	38.1
236	476	ATTTTCTTGTCTGTAAT	28.8	26.2
237	477	AAAACAAGAGTTGGAAGAGC	45.4	35.2
238	478	ATCTTGTTCAAAACAAGAGT	40.2	32.1
239	479	CTTGCAATTTTTCACGGTCA	51.2	37.7
240	480	TTCTTGCAATTTTTCACGGT	50.0	37.7
241	481	TCTTCAACAGCTGCACGAGT	50.8	36.3
242	482	ACCTTCTTCAACAGCTGCAC	49.3	36.1
243	483	AACAATACCTTCTTCAAC	35.0	29.1
244	484	CACCTGCAACAATACCTTCT	46.7	35.3
245	485	CCACCACCTGCAACAATACC	49.1	36.4
246	486	TTGTGAGCAATTTGACGAAC	48.5	35.6
247	487	TACAGTTGACAAAGAGCCTA	42.4	32.9
248	488	CTACAGTTGACAAAGAGCCT	43.5	33.6
249	489	ACTACAGTTGACAAAGAGCC	43.2	33.3
250	490	CCCACTACAGTTGACAAAGA	44.7	33.6
251	491	ACCCACTACAGTTGACAAAG	43.8	33.3
252	492	CAACCCACTACAGTTGACAA	45.4	33.6

Table 3: Antisense Oligonucleotides that Target *Streptococcus pyogenes groEL*

SEQ ID No:	Name	Sequence 5 → 3'	Tm (°C)	ΔG (kcal/mol)
253	493	ATCATTGGTTTTAGAAGCC	43.4	34.2
254	494	ATATCATTGGTTTTAGAAGC	40.8	33.5
255	495	TCGTCCCATCACCAGCAATA	53.6	38.5
256	496	GCAGTAGTCGTCCCATCACC	51.7	37.3
257	497	TGTTTCAATGCCTCGACGGA	56.3	40.0
258	498	GCTGTTTCAATGCCTCGACG	55.6	40.0
259	499	CTGTTGCTGTTGCTGTTTCA	49.6	35.5
260	500	CGGCTTTCAAGGCTTCAACA	55.4	40.5
261	501	GCAACGGCTTTCAAGGCTTC	56.4	41.7
262	502	TTGCCAGATACAGGTTGAGC	50.2	36.8
263	503	GCAGCGACCTGAGCAATAGC	55.1	40.8
264	504	TCCAACTTTTTCAGAGCGTG	50.5	37.2
265	505	GTTGCCCACACGCTCCATAG	55.3	39.7
266	506	CACACCATCGTTGCCCACAC	56.7	38.9
267	507	AGTTCTGTTTCCATACCTCG	45.9	35.2
268	508	TCAAGTTCTGTTTCCATACC	43.9	33.8
269	509	TCAACCACTTCAAGTTCTGT	44.0	33.1
270	510	CCTTCAACCACTTCAAGTTC	46.0	34.9
271	511	TAACCACGGTCAAATTGCAT	50.5	37.5
272	512	ACCATTTTTTCATTGTCTGT	43.5	33.8
273	513	GCAACCATTTTTTCATTGTC	47.2	35.9
274	514	AATGGGTTTTCAAGGTCTGC	50.8	38.1
275	515	TTTTATCCATAATTAAGAT	33.5	30.0
276	516	TCCTCAAGTAGTGGCAAAAT	46.5	35.6
277	517	AGTAATGGACGGTTGGTTTT	48.0	37.0
278	518	AATGAGTAATGGACGGTTGG	49.0	36.9
279	519	TCACCATCCACATCATCTGC	51.1	35.8
280	520	AGGGCTTCACCATCCACATC	53.5	38.6
281	521	GGAAGGGCTTCACCATCCAC	55.5	40.2
282	522	AAGACAAGGGTTGGAAGGGC	54.5	40.6
283	523	GTACCACGAATCTTGTTCA	42.4	32.1
284	524	TTACGACGATCACCAAATCC	49.9	37.1
285	525	AGCTTTACGACGATCACCAA	49.9	37.2
286	526	GCTTTACGACGATCACC	44.5	33.4

SEQ ID No:	N[redacted]	Sequence 5 → 3'	[redacted]m (°C)	ΔG (kcal/mol)
287	527	ACCACCAGTCAAGATAGCAA	46.9	35.0
288	528	GTCATTGTAGCATCTTTTA	40.6	32.1
289	529	CTGTCATTGTAGCATCTTT	44.3	34.0
290	530	TCTTGTAGTTTTTCACGGT	44.2	34.4
291	531	CCAGCTAATTTCGCCAAACG	55.0	41.4
292	532	CACCAGCTAATTTCGCCA	53.6	39.0
293	533	CACCACCAGCTAATTTCGCC	54.6	40.3
294	534	GATAACAGCTACACCACCAG	44.4	33.7
295	535	CCTACTTTGATAACAGCTAC	38.5	31.8
296	536	GTGTGGCATTTAGAGCATCC	49.6	36.7
297	537	CCTTCTTCAACGGCTGCACG	58.0	41.6
298	538	GATACCTTCTTCAACGGCTG	49.0	37.0
299	539	GCAACGATACCTTCTTCAAC	46.5	35.5
300	540	CCAGCAACGATACCTTCTTC	49.0	37.0
301	541	CCACCACCAGCAACGATACC	47.5	36.0
302	542	GTTCCACCACCAGCAAC	45.5	32.2
303	543	CCGTAATAAGTGCTGTTCCA	48.0	36.3
304	544	CGCCCTCAAGCTCAAGAGCA	57.9	41.4
305	545	TCATCGCCCTCAAGCTCAAG	55.3	39.8
306	546	CGTCATCGCCCTCAAGCTCA	58.4	41.2
307	547	AGCCTTCGTACCCAGCATTT	53.0	40.0
308	548	CGGAGCCTTCGTACCCAGCA	59.7	43.0
309	549	CAATAACTACGGAGCCTTCG	49.5	38.0
310	550	TGTCAATAACTACGGAGCCT	46.6	35.7
311	551	AGAAGCTGCATTTTGAAG	42.4	32.5
312	552	GCTACAGAAGCTGCATTTTG	48.0	36.2
313	553	ACCTGCTGGCATTGCTGGCG	62.8	44.3
314	554	TTAGAAGCCGCCCATCATTC	54.2	40.5
315	555	AAAAAAACAAAGATGGGATA	43.7	35.2
316	556	ACTAAAAAAACAAAGATGGG	43.4	35.0
317	557	ATTTCCCTCCAACACCCATA	50.8	38.2
318	558	AGCCTTTGCGACATCTTTTA	49.6	37.9
319	559	CCAGCCTTTGCGACATCTTT	54.3	40.1
320	560	GTTGATACACCAGCCTTTGC	49.7	36.8
321	561	AGTCCGTCTCCCCTTTTTCG	54.7	41.0
322	562	TCATCCAAAATCAGCATAAA	45.8	35.1

SEQ ID No:	Na[redacted]	Sequence 5 → 3'	[redacted]m (°C)	ΔG (kcal/mol)
323	563	AATGCCTCACTTTTGGATAA	46.7	36.1
324	564	TGTTACGAAATGCCTCACTT	47.4	35.8
325	565	TTGAGTTGTTACGAAATGCC	47.9	36.1
326	566	TCCAGCCAGAAGGTAAAGTC	48.5	36.6
327	567	ACAACACGATAACTTCCCAG	46.5	35.2
328	568	AAAGGTGGTGAATCTGGTGA	49.1	36.0
329	569	CTAAAAGGTGGTGAATCTGG	46.4	35.6
330	570	TTTTACCCCTTTTTTCTTAT	42.9	35.8
331	571	AACTCTTTTACCCCTTTTTT	44.1	36.3
332	572	CCAAAAAAACAACAAAGAAA	45.2	35.5
333	573	TAAGCCAAAAAAACAACAAA	45.9	36.0
334	574	CGGGTTGAGATAGCGTTAGT	48.8	37.4
335	575	GGGGTGAATAAGATGGGAAT	49.5	38.1
336	576	TATAGGAGGGGTGAATAAGAT	43.8	35.3
337	577	CACTTTGTGAGACCTTATCGG	45.9	35.2
338	578	AATACCATAACCCAAAAAAGC	45.7	36.6
339	579	CCAGCAATATCATTGGTTTT	47.0	36.3
340	580	AACAGCTGTTGCTGTTGCTG	51.4	36.7
341	581	CAACAGCTGTTGCTGTTGCT	51.4	36.7
342	582	ATGGTAATCACACCATCGT	44.2	33.1
343	583	AATTGCATGCCTTCAACCAC	52.4	38.3
344	584	AGGTAACCACGGTCAAATTG	49.1	37.0
345	585	TTTATCCATAATTAAGATAA	34.5	30.8
346	586	CCACGAATCTTGTTCAA	40.7	30.5
347	587	CGACGATCACCAAATCCTGG	55.1	39.6
348	588	GCTTTACGACGATCACCAAA	50.4	37.5
349	589	AGCATAGCTTTACGACGATC	46.6	36.1
350	590	TCTTCAAGCATAGCTTTACG	45.4	35.4
351	591	ATAACAGCTACACCACCAGC	46.8	35.2
352	592	GCCGCCCATCATTCCTGGAT	60.4	43.8
353	593	AGCCGCCCATCATTCCTGGA	61.1	43.9
354	594	CTCTAAAGGTTTTAAAAAT	35.6	31.2

Table 4: Antisense [redacted]onucleotides that Target *Staphyloc[redacted]s aureus groE* operon

SEQ ID No:	Name	Sequence 5 → 3'	Tm (°C)	ΔG (kcal/mol)
355	595	ACCGCTTCTCATTGTAAAAA	47.0	35.4
356	596	ATTTTTACCGCTTCTCATTG	46.7	36.6
357	596a	TTAGCATGATTGTTCCTCCT	55.6	35.6
358	596b	TTTCCAATTGGTTTTAGCAT	56.4	36.8
359	597	TTTTTTCTCAATAATCACAC	39.3	31.9
360	598	TCTTTTTTCTCAATAATCAC	38.7	31.9
361	599	TTCTTGTTCTTTTTTCTCA	39.5	31.5
362	600	CGCCTTCGTTTGATTTTTCT	51.4	39.4
363	601	ACGGTCCCCTTCTTTCACTT	51.8	39.0
364	601a	CCATAAATGATAAACCTCCA	53.8	36.2
365	601b	AATTTCAATTGTTTAACCAT	50.5	33.7
366	602	ATCGTTACACCATCGTTCGT	49.4	36.7
367	603	GCAATCGTTACACCATCGTT	50.0	37.1
368	604	CCTTCTTGAATCATTGCTTG	47.4	35.9
369	605	GCACCACTTGTAACATTTTT	44.5	34.4
370	606	ATTTCATTTTTATTTTCAAC	38.7	32.7
371	607	ATTTCATTTTTATTTTCAA	37.0	31.4
372	608	TGCTGAAATCGCACCTACTT	50.1	37.3
373	609	TCATCTGCTGCTGAAATCGC	53.8	38.5
374	610	ATCGTTACCTACTTTTTCCG	45.8	36.5
375	611	CATCGTTACCTACTTTTTCC	43.6	34.8
376	612	CCATCGTTACCTACTTTTTC	43.6	34.8
377	613	GTTACTAAAATGTATGGGC	38.9	31.9
378	614	ACTTGTTCTAATAAAGGTAA	35.7	30.9
379	615	ACTTGTTCTAATAAAGGTAA	35.7	30.9
380	616	CGATTAGATTGAACCACTTG	44.7	34.5
381	617	TTGGACGATTAGATTGAACC	46.7	35.7
382	618	ATTGGACGATTAGATTGAAC	43.2	34.1
383	619	TCGCCTTCAACTTCATCAGC	52.8	38.3
384	620	TGCATCGCCTTCAACTTCAT	53.1	38.5
385	621	CACGCATTCGGTTTAGCACG	56.5	41.0
386	622	TGCCACGCATTCGGTTTAGC	58.3	42.3
387	623	TAACATTGCTTTACGACGAT	45.3	35.2
388	624	TCTTCTAACATTGCTTTACG	42.7	33.9

SEQ ID No:	Na[redacted]	Sequence 5 → 3'	[redacted]Tm (°C)	ΔG (kcal/mol)
389	625	TCGTCACCGTCACCATCAAC	53.8	37.3
390	626	TCAATGCTGTTTTCGTCACC	50.9	36.8
391	627	ATACCTTCTTCAACTGCTGC	46.2	35.3
392	628	ACAATACCTTCTTCAACTGC	43.4	33.8
393	629	GCAACAATACCTTCTTCAAC	43.9	34.1
394	630	CACTCGTTTGTAGCACCGTT	50.3	37.0
395	631	GCTACAACCGCTTCAGTCGT	52.0	38.2
396	632	TGATGCTACAACCGCTTCAG	51.3	37.3
397	633	GTCGTTTTACATCATTCCCG	49.6	37.4
398	634	AAAAGACGGTTCCCAAAAGT	49.7	38.4
399	635	TACGGGCTCATACAAAAAAG	47.5	36.9
400	636	ATTTTAATTAATTTAAAAAT	33.9	31.5
401	637	TTTGGAGGAAAATTATGA	39.8	31.7
402	638	GTTATTTACGATAAAATAA	32.0	29.2
403	639	TCAATAATCACACGATTTCC	44.8	34.4
404	640	TTCTCAATAATCACACGATT	41.9	32.9
405	641	TTGTACCATCATTTAATA	32.0	27.8
406	642	ATTTAATTTTATATTATTC	28.3	27.9
407	643	CCACGTAACATTGCTTGACG	51.6	37.5
408	644	ACACCACGTAACATTGCTTG	48.3	35.5
409	645	CAACATTACGTCCTTTAGG	42.2	33.2
410	646	ATCTTCTAATTCGATTTC	38.7	31.3
411	647	CCCATATTTTCATATGGATC	43.7	34.8
412	648	TTCAAGCCTTCTTGAATCAT	46.5	35.6
413	649	TTTTTCAAGCCTTCTTGAAT	46.3	36.3
414	650	TTTTTCAAGCCTTCTTGA	42.9	32.9
415	651	ACTGGGTTCGCACCACTTGT	54.2	38.7
416	652	CCAACTGGGTTCGCACCACT	57.1	40.5
417	653	TGACGTAAACCAACTGGGTT	49.5	36.8
418	654	CCTTGACGTAAACCAACTGG	49.7	37.1
419	655	GCTTTGTCGATACCTTGACG	49.9	37.2
420	656	ACTGCTTTGTCGATACCTTG	46.9	35.5
421	657	TAACTGCTTTGTCGATACC	42.5	32.9
422	658	TATTTTCAACTTTTTGAGAA	39.7	32.6
423	659	TTATTTTCAACTTTTTGAGA	39.7	32.6
424	660	TTATTTTCAACTTTTTGAG	37.2	31.0

SEQ ID No:	Na[redacted]	Sequence 5 → 3'	[redacted]m (°C)	ΔG (kcal/mol)
425	661	TTTTTATTTTCAACTTTTTG	40.2	33.5
426	662	ATTTCATTTTTATTTTCAAC	38.7	32.7
427	663	TCTTCATCTGCTGCTGAAAT	47.3	35.3
428	664	ATTTCTTCATCTGCTGCTGA	47.3	35.3
429	665	CCAATTTCTTCATCTGCTGC	49.9	37.1
430	666	CGTCCAATTTCTTCATCT	41.5	32.0
431	667	TTTGATTCTTCAATTGTAAT	39.1	32.1
432	668	ATTTGATTCTTCAATTGTAA	39.1	32.1
433	669	TAACCACGATCAAATTGCAT	48.1	36.2
434	670	GATAACCACGATCAAATTGC	47.1	35.9
435	671	GATTGATAACCACGATCAAA	44.8	34.4
436	672	GGTGATTGATAACCACGATC	46.5	35.0
437	673	TATGGTGATTGATAACCACG	45.6	34.6
438	674	ATATATGGTGATTGATAACC	38.0	31.7
439	675	ACCATATATGGTGATTGATA	38.9	31.7
440	676	GTAACCATATATGGTGATTG	39.2	31.8
441	677	GAATCAGTAACCATATATGG	38.5	31.8
442	678	TTATCTGAATCAGTAACCAT	38.4	31.4
443	679	CATTTTATCTGAATCAGTAA	36.8	30.8
444	680	TAATTCAGCAACCATTTTA	40.9	32.6
445	681	TCTAATTCAGCAACCATTTT	44.4	34.9
446	682	AAAGGTAAGATATCTTGGAA	40.2	33.3
447	683	ACTTGTTCTAATAAAGGTA	32.9	29.0
448	684	TGAACCACTTGTTCTAATAA	39.7	31.9
449	685	TTGAACCACTTGTTCTAATA	39.7	31.9
450	686	GTTAATGCATCGCCTTCAAC	49.9	37.5
451	687	TTTGTTAATGCATCGCCTTC	50.6	38.1
452	688	ATATTTGTTAATGCATCGCC	47.3	36.9
453	689	ACGATATTTGTTAATGCATC	42.0	33.6
454	690	GTAAATGTGCCACGCAT	43.9	32.1
455	691	CCGAAACCAGGCGCCTTTAC	58.3	43.6
456	692	TCACCGAAACCAGGCGCCTT	61.2	44.3
457	693	CGACGATCACCGAAACCAGG	57.5	41.1
458	694	GCTTTACGACGATCACCGAA	52.8	38.9
459	695	ATTGCTTTACGACGATCACC	49.3	37.1
460	696	CATTGCTTTACGACGATCAC	48.2	35.9

SEQ ID No:	Na[redacted]	Sequence 5 → 3'	[redacted]m (°C)	ΔG (kcal/mol)
461	697	AACATTGCTTTACGACGATC	47.0	35.9
462	698	TGTAATTTTTCACGATCAAA	43.4	33.9
463	699	TCTTGTAATTTTTCACGATC	41.9	33.3
464	700	GCGCTCTTGTAATTTTTC	42.5	33.2
465	701	AAGCGCTCTTGTAATTTTTC	46.3	36.7
466	702	GCTAAGCGCTCTTGTAATTT	46.5	36.9
467	703	CCACCTGCTAATTTAGCTAA	44.7	35.7
468	704	TTAATAACTGCAACACCACC	45.2	34.6
469	705	ACTTTAATAACTGCAACACC	41.3	33.1
470	706	TGCACCTACTTTAATAACTG	40.0	32.4
471	707	AACAATACCTTCTTCAAC	35.0	29.1
472	708	CCACCACCTGCAACAATACC	52.6	38.0
473	709	TTTAAGTACAATATTTACA	29.5	27.3
474	710	CAATTTGACGAACTGGTGCA	51.8	37.1
475	711	CAGCAATTTGACGAACTGGT	50.1	36.8
476	712	TTTTCAGCAATTTGACGAAC	48.0	36.2
477	713	CCTGCATTTTCAGCAATTTG	51.0	38.0
478	714	AATCCTGCATTTTCAGCAAT	49.1	37.3
479	715	TCTAATCCTGCATTTTCAGC	47.2	36.1
480	716	GTTACTTTAGTTGGATCAA	36.1	30.0

Table 5: Conservation of Antisense Oligonucleotides targeting *groE* Operon of *E. coli* among Microorganisms

GENUS/SPECIES	Antisense Oligonucleotide Name																				
	269	280	284	285	288	291	293	294	295	296	297	298	299	300	302	305	307	311	312	313	314	315
Aeromonas salmonicida																						
Bartonella															Y							
Bacillus															Y							
Bordetella pertussis																						
Brucella					Y																	
Campylobacter jejuni																						Y
Chlamydia																		Y	Y	Y	Y	Y
Coxiella burnetii																	Y	Y	Y	Y	Y	Y
Enterobacter	Y	Y	Y	Y		Y	Y	Y	Y	Y	Y	Y	Y	Y				Y				
Erwinia	Y		Y	Y	Y									Y				Y				
Escherichia coli	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Haemophilus influenzae																		Y	Y	Y	Y	Y
Helicobacter pilori																						
Klebsiella		Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y			Y	Y	Y	Y	Y	Y
Legionella																		Y				
Leptospira interrogans																						
Mycobacterium																						
Neisseria						Y	Y	Y														
Pseudomonas					Y	Y	Y	Y				Y	Y									
Ricketsia								Y														
Salmonella		Y	Y	Y	Y	Y	Y	Y				Y	Y	Y		Y	Y	Y				

GENUS/SPECIES	Antisense Oligonucleotide Name																					
	269	280	284	285	288	291	293	294	295	296	297	298	299	300	302	305	307	311	312	313	314	315
Serratia	Y					Y	Y	Y	Y	Y	Y			Y				Y				
Sitophilus	Y		Y	Y																		
Staphylococcus																						
S. maltophilia	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	
Streptococcus pyogenes																						
Streptomyces																						
Yersinia enterocolitica				Y	Y	Y	Y	Y	Y	Y	Y	Y	Y					Y	Y	Y	Y	Y

Table 6: Antisense oligonucleotides targeting the mRNA of the *groE* operon of *S. pneumoniae* that are conserved among microorganisms

GENUS/SPECIES	Antisense Oligonucleotide Name																		
	415	423	429	430	431	432	433	435	436	456	457	458	459	466	467	468	469	470	471	472
Acyrthosiphon pisum						Y														
Amoeba proteus								Y												
Arabidopsis thaliana														Y						
Bacillus														Y			Y	Y		
Bartonella		Y						Y		Y	Y	Y	Y							
Borrelia burgdorferi															Y					
Buchnera aphidicola						Y	Y													
Clostridium																				Y
Campylobacter jejuni																				
Candida albicans											Y									
Chlamydia muridarum						Y														
Haemophilus influenzae									Y											
Helicobacter pylori										Y	Y									
Lactobacillus																			Y	
Legionella pneumonphila					Y	Y														
Mycoplasma genitalium	Y															Y				
Neisseria gonorrhoeae										Y	Y									
Onchocerca volvulus										Y	Y	Y								
Plasmodium falciparum						Y														
Rhopalosiphum padi			Y	Y	Y	Y							Y		Y					
Staphylococcus																				

GENUS/SPECIES	Antisense Oligonucleotide Name																			
	415	423	429	430	431	432	433	435	436	456	457	458	459	466	467	468	469	470	471	472
Streptococcus agalactiae																				
Streptococcus gordonii																				
Streptococcus iniae																				
Streptococcus pneumoniae	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Streptococcus pyogenes		Y																		

Table 7: Antisense oligonucleotides targeting the *groEL* mRNA of *S. pyogenes* that are conserved among microorganisms

GENUS/SPECIES	Antisense Oligonucleotide Name																			
	493	494	508	509	510	511	512	513	515	523	524	525	526	528	529	530	532	534	535	537
Actinobacillus						Y														
Acyrthosiphon pisum											Y		Y							
Arabidopsis thaliana						Y														
Bacillus						Y							Y							
Bartonella		Y									Y		Y					Y		
Borrelia														Y	Y					
Cyanophora paradoxa													Y							
Escherichia coli																				
Haemophilus influenzae													Y							
human immunodeficiency virus																				
Klebsiella									Y				Y							
Lactobacillus						Y				Y	Y	Y	Y					Y		
Legionella micdadei													Y							
Pisum sativum						Y														
Pseudomonas putida																				Y
Simian immunodeficiency virus																				
Staphylococcus					Y	Y							Y						Y	
Streptococcus iniae			Y	Y	Y		Y	Y												
Streptococcus pneumoniae		Y																		
Streptococcus pyogenes	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Synechocystis	Y																			
Thermotoga maritima																	Y			

GENUS/SPECIES	Antisense Oligonucleotide Name																		
	493	494	508	509	510	511	512	513	515	523	524	525	526	528	529	530	532	534	535	537
Zymomonas mobilis												Y	Y							

Table 8: Antisense oligonucleotides targeting the mRNA of the *groE* operon of *S. aureus* that are conserved among microorganisms

GENUS/SPECIES	Antisense Oligonucleotide Name																			
	597	598	599	603	604	605	606	607	611	612	613	614	615	616	617	618	619	623	624	627
Actinobacillus																				
Acyrthosiphon pisumendosy																				
Amoeba proteus																				
Arabidopisi thaliana																				
Bacillus			Y																	
Borrelia burgdorferi											Y									
Caenorhabacter																				
Campylobacter																				
Clostridium																				
Cyanophora paradoxa																				
Ehrlichia																				
Escherichia coli																				
Heliobacterium								Y				Y								
Klebsiella																				
Lactobacillus				Y																
Legionella																				
Pasteurella multocida																				
Plasmodium falciparum								Y												
Rickettsia																				
Shigella																				
Staphylococcus	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Streptococcus																				
Streptococcus																				

GENUS/SPECIES	Antisense Oligonucleotide Name																			
	597	598	599	603	604	605	606	607	611	612	613	614	615	616	617	618	619	623	624	627
pneumoniae																				
Synechocystis	Y	Y																		
Treponema pallidum																				
Yersinia enterocolitica																				

Table 9: Primers used to PCR amplify the *groE* operon.

PATHOGEN	FRAGMENT 1		FRAGMENT 2		FRAGMENT 3	
Primer	Forward 5'→ 3'	Reverse 5'→ 3'	Forward 5'→ 3'	Reverse 5'→ 3'	Forward 5'→ 3'	Reverse 5'→ 3'
Escherichia coli,	TAA CAG ATA CGG ACT TTC TC [SEQ ID NO:481]	AGA TTT TCT TGT CAG CCA GCA [SEQ ID NO:486]	TCC TTA CTT CAT CAA CAA GCC [SEQ ID NO:491]	GTT TAT TTC TGC GAG GTG CA [SEQ ID NO:495]		
Streptococcus pneumoniae	CAG ATG TTG AAA CCA TTA GGG [SEQ ID NO:482]	TGT ACT GTG AAA GGT AAC CAC [SEQ ID NO:487]	ACG TGG TAT GGA AAC AGA GC [SEQ ID NO:492]	TTA CAT CAT CCC GCC CAT C [SEQ ID NO:496]	GAT TTG GTC TTG GAA GAG G [SEQ ID NO:499]	TTT GAC ATA CTT ACT TTC TCC [SEQ ID NO:501]
Streptococcus pyogenes	GAA GCT ATG GAG CGT GTG G [SEQ ID NO:483]	CGT TCT TGT AGT TT TCA CGG [SEQ ID NO:488]	GTA ATT GTT GAA GGT TCA GG [SEQ ID NO:493]	CAT TAA AAG TCT GTA ACC ACA [SEQ ID NO:497]		
Staphylococcus aureus	CAA TCA TGC TAA AAC CAA TTG G [SEQ ID NO:484]	GAA TCA GTA ACC ATA TAT GGT G [SEQ ID NO:489]	GGT ATG CAA TTT GAT CGT GGT [SEQ ID NO:494]	TTC CCG GCA TGC CAC CCA [SEQ ID NO:498]		
Pseudomonas aeruginosa	ATG AAG CTT CGT CCT CTG [SEQ ID NO:485]	CGT TCT TGT AGT TTT TCA CGG [SEQ ID NO:490]			AAA CAC CAC CAT CAT CGA TG [SEQ ID NO:500]	GCT TCG GTG GTG ATC ATC [SEQ ID NO:502]